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Polyherbal Nanogel: A Potential Remedy For The Management Of Rheumatoid Arthritis

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Abstract:

Due to reports that these medicines are secure and have minimal unfavourable side effects, particularly when compared to produced pharmaceuticals, interest in using medicinal plants has gradually grown in poor countries in recent years. In the present study, simple, efficient, and economical silver nanoparticles (AgNPs) were produced using the leaf extracts of the three herbs Vitex negundo, *Ehretia laevis* and rhizomes of *Curcuma amada*. This multi-herb extract was transformed into synthetic AgNPs, which were more active than the original extract. After synthesising the polyherbal extract and its AgNPs the nanogel was made using a few gelling agents, and the resulting silver nanoparticles nanogel formulation was evaluated. The anti-arthritic characteristics of the recently discovered topical polyherbal nanogel formulation may be due to luteolin, which is present in methanol extracts of polyherbal silver nanoparticles. It was found that the newly developed formulation F2, which contains 1.5% of carbopol 934 and 2% of the three herb extracts, is a potential topical polyherbal nanogel for the management of arthritis. The effectiveness of this formulation for persons with inflammatory joint conditions may be further supported by clinical studies.

Keywords: *Vitex negundo*, *Ehretia laevis*, *Curcuma amada*, Polyherbal, Rheumatoid arthritis, Nanogel, etc.

Introduction

For the treatment of numerous illnesses, more than 80% of the world's population still heavily relies on traditional remedies [1]. Because herbal medicines have been reported to be safe and to have substantially fewer undesirable side effects than synthetic pharmaceuticals, especially in impoverished countries, there has been a steady resurgence of interest in the use of medicinal plants in recent years [2]. Due to their widespread usage and unclear benefit/risk ratio, topical herbal therapies have drawn a lot of interest [3]. In comparison to cream and ointment, topical administration of gels at desired locations gives substantial advantages in a faster release of a medicine straight to the site of action [4].

Chronic inflammation brought on by synovial hyperplasia characterises rheumatoid arthritis, an inflammatory condition that worsens with significant, permanent bone loss. Other signs and symptoms include skeletal, cardiovascular, pulmonary, and pulmonary physiological abnormalities as well as stiffness and lack of physical movement. Rheumatoid arthritis (RA), which has a substantial impact on quality of life, affects around 1% of individuals worldwide, according to a recent epidemiological study. In all populations, women are more likely to have it than men. In over 80% of cases, RA begins to manifest itself between the middle of the fourth and the end of the fifth decade of life. Treatment options for RA include medications and lifestyle modifications. Nonsteroidal anti-inflammatory medicines (NSAIDs), such as salicylic acid, and steroids (usually cortisone injection) are used as current treatments. Although these medications reduce pain, they cannot restore damaged tissues. No medication is known to entirely cure RA, even though a wide variety of medications are administered to manage the pain and limit the disease's progression. Additionally, taking NSAIDS often and taking steroids that suppress the adrenal glands have been linked to stomach ulcers in RA patients. Patients are frequently forced to seek out complementary and alternative medicine (CAM) due to these unfavourable side effects [5].

According to a recent poll, those with RA who experience chronic pain and who are unsatisfied with allopathic treatment are more likely to turn to alternative medicine, which is used by 60–90% of people with arthritis. Finding a feasible substitute to the current allopathic medical system would therefore be highly desirable. Since ancient times, natural plant compounds have been used to treat and prevent a variety of ailments. According to a World Health Organisation (WHO) survey, traditional medicine is used by nearly 80% of the global population. Nearly 90 of the 121 medications that are currently prescribed in the USA are derived directly or indirectly from plants. Herbal medicines can serve as an alternate source to treat the symptoms of RA patients as well as solve the problems with current allopathic

drug-based therapy regimens. Vitex negundo, *Ehretia laevis* and *Curcuma amada* have a crucial function in reducing the excruciating pain and inflammation associated with RA, among all researched herbs, it is scientifically evident [6,7].

Material and Methods

Materials

Leaves of *Vitex negundo*, *Ehretia laevis* and rhizomes of *Curcuma amada* fresh part were gathered and verified. Sigma-Aldrich was used to acquire Freund's complete adjuvant (FCA), diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate. Carbopol 934 and carbopol 940 were purchased from Mumbai's Loba Chemie Pvt. Ltd.

Preparation of extracts

Leaves of Vitex negundo, *Ehretia laevis* and rhizomes of *Curcuma amada* were cleansed, and any leftover materials and earthy residue were carefully removed. They were then allowed to dry in the shade. All three herbs were coarsely ground, and methanol was extracted using a 7-day cold maceration process. After being filtered and concentrated under reduced pressure in an IKA Rotary evaporator (Model No RN 10 digital V, ILMAC Germany), the extracts were then held at 4–8 ^{o}C for subsequent use.

Animals

Wistar strain rats (12-week-old healthy, weighing 150-200 g of either sex in the animal house of Pharmacy college, Nagpur, India) were chosen for the anti-arthritic assessment., while albino rabbits with an average weight of 2.2 kg were used for the primary skin irritation test. They were housed in climate-controlled conditions with 10- to 14-hour cycles of light and dark, temperatures of 23.2 oC, and a relative humidity of 50.5%. The animals were housed in individual polypropylene cages with sterile rice husk bedding and unrestricted access to food and drink. The experiment designs and procedures were approved by the Institutional Animal Ethical Committee and the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Preliminary phytochemical investigations [8-11]

The main secondary metabolites, such as alkaloids, flavonoids, saponins, phenols, terpenoids, protein and amino acids, carbohydrates, and glycosides, were assessed using the standard method Harborne described. There were standard operational procedures applied.

Phytochemical analysis of plant extracts

Each stock concentration of 1% (W/V) was prepared using the appropriate solvent. All three herb extracts were combined, along with positive and negative controls, to test for the presence of active phytochemicals. Preliminary phytochemical screening was used to identify the presence of alkaloids, flavonoids, tannins, carbohydrates, phenolic compounds, terpenoids, glycosides, steroids, fixed oils, and fats in the extracts of all three herbs. The presence of the phytochemical components was typically assessed by adding the appropriate chemical reagent(s) to the mixture of extracts from all three herbs in test tubes.

Test of Alkaloid

Mayer’s test

Alkaloids are common nitrogenous compounds with different physiological and pharmacological effects. Mayer's reagent, which causes the addition of a few drops of alkaloid solution to produce a white yellowish precipitate, is used to precipitate most alkaloids from neutral or slightly acidic solutions. The extract of all three herbs in an alcoholic solution was dried by evaporation before the residue was boiled in a bath of boiling water with 2% hydrochloric acid. After cooling, the mixture was filtered and given a Mayer's reagent treatment. The samples were then examined for turbidity or yellow precipitation after that.

Test of Flavonoids

Lead Acetate Test

Several drops of a 10% lead acetate solution were used to treat the extracts. The presence of flavonoids is indicated by the precipitate's yellow coloration.

Test of Glycosides

Aqueous Sodium hydroxide

With 1 ml of water and 1 ml of sodium hydroxide, extracts were processed. The development of a yellow hue denotes the presence of glycosides.

Test of Steroids
Salkowski Test
To treat the extracts, a few drops of strong sulphuric acid and chloroform were applied. When the chloroform layer's green fluorescence acid layer turns blue red to cherry in colour, steroids are present.

Test of Terpenoids
5 ml of each extract were mixed with 2 ml of chloroform and 3 ml of strong sulphuric acid to produce a reddish-brown monolayer at the interface. The terpenoids benefited from this, which had a positive impact.

Test of Tannins
When 0.1% ferric chloride solution was added to the extract, a dark blue or greenish black colour resulted, indicating the tannins were present.

Test of Phenols
Ferric Chloride Solution
Three to four drops of a 5% ferric chloride solution were added to the extracts. Phenols are present when a bluish black colour forms.

Detection of carbohydrate
Each extract was individually diluted in 5 cc of distilled water and then filtered. The presence of carbohydrates was examined in the filtrates.

Benedict's test
Benedict's reagent was applied to the filtrates, and they were gently heated in a water bath for ten minutes. Precipitation that is brick red suggests the presence of reducing sugars.

Synthesis of mixture of all three herbal extract loaded silver Nanoparticles
In the one-step green synthesis, 25 ml of extract was combined with 95 ml of a 1 mM aqueous silver nitrate solution, which was then left at room temperature and in the dark for 24 hours. Silver nanoparticles are produced when pure silver ions are reduced, and this process was seen by determining the absorbance of the reaction medium in the wavelength range of 300-700 nm using UV spectrophotometry. The generated silver nanoparticles (AgNPs) were cleaned using centrifugation for 15 minutes at 1000 rpm. For further particle settlement, the supernatant was transferred to a clean, dry beaker. The AgNPs were then centrifuged using a cooled microfuge, dried, purified, and characterised.

Characterization of silver nanoparticles
UV-Vis Spectroscopy Analysis
Studies of the UV-visible spectrum of Ag nanoparticles generated in green synthesis were conducted using quartz cells in a UV-vis spectrophotometer (Lark, model: LI-UV-7000) between the wavelength range of 200-700 nm. Double-distilled water was used as a blank. The characteristic peak of Ag nanoparticles is projected to be at 440 nm.

Fourier transform infrared (FTIR) spectral analysis
The infrared spectra of plant extract and made AgNPs were acquired for the purpose of identifying functional groups in a (Perkin Elmer Spectrum 2, Germany) spectrophotometer IR affinity-1 by utilising the KBr pellet technique and registering amplitude waves with a range of 400 to 4000 cm-1.

Particle Size and Zeta Potential Analysis
The zeta potential and particle size distribution were measured using the Malvern Zeta sizer Instrument Ltd, which is based on the DLS method. Using a solution of manufactured nanoparticles dispersed in 2 mL of distilled water, the zeta potential was measured as well as the size distribution was examined.

Drug entrapment efficiency (DEE)
After centrifuging silver nanoparticles at 15,000 rpm for 40 min, the supernatant was collected, filtered through a 0.22 micron membrane filter, and the amount of drug contained was assessed using a UV-Visible spectrophotometer at a particular wavelength. Using the equation $y = 0.0164x + 0.0076$, ($R2 = 0.996$), where y stands for absorbance and x stands for concentration (mcg/ml), the amount of medication in the supernatant was determined. DEE was computed by subtracting the amount of drug added overall from the amount of drug contained in the supernatant.

Encapsulation efficiency (%) =

$$\frac{\text{Total drug(mg)} - \text{Free drug (mg)} \times 100}{\text{Total drug (mg)}}$$

Transmission electron microscopy (TEM)
The AgNPs size and shape were examined using transmission electron microscopy (TEM) in Jeol, Japan. The microscope was accelerated at an 80 kV voltage. The silver samples were diluted in distilled water (1:10) before a 20-L aliquot was applied on a grid that had been dusted with carbon. The excess solution was left on the grid for one minute, and then it was wiped using filter paper. The grids were allowed to dry in the grid box for two hours before imaging.

In-*vitro* drug release study
The USP standard type II dissolution test apparatus was used to conduct the in vitro dissolution research of the formulation of silver nanoparticles. By placing the study's formulation quantity in a muslin cloth and placing it in 900 ml of dissolution media circulated at 50 rpm and maintained at 370.5 C, the study was carried out in pH 7.4 phosphate buffer solution. At 15, 30, 45, 60, 75, and 90 minutes, 5 ml aliquots of the dissolving medium were taken out, accordingly. In the meantime, the same medium was refilled in an equal amount. Samples of dissolution were filtered via 0.22 m filters and spectrophotometrically evaluated at a certain wavelength.

Preparation of gel base
Carbopol 934 was slowly dissolved while being agitated in 60 mL of demineralized water for an hour to prevent agglomeration. The next step was to individually dilute triethanolamine and disodium edetate in 10 mL of demineralized water before stirring for 10 minutes. 4.83 mL of propylene glycol and 12 mL of demineralized water were mixed and agitated for 10 minutes. After adding triethanolamine solution and disodium edetate, the mixture was stirred for 10 minutes to bring the pH of the carbopol solution to 7.4. Propylene glycol solution was added after whirling for 10 minutes to produce a transparent, homogenous gel base.

Preparation of polyherbal nanogel formulation
Six topical nanogel formulations were made utilising a combination of all three herbs and a methanol extract of silver nanoparticles, according to the medication formulation instructions. The gel base of carbopol 934 (1.5%) was used to develop formulations F1 to F6. Details of the formulation component are presented in Table I. The F4 formulation manufactured with carbopol 934 was examined for its capacity to treat arthritis because of its improved quality qualities.

Table 1: Polyherbal Nano Gel formulations with carbopol 934

Nanogel code	All three herbs extract loaded silver nanoparticles (g)	Carbopol 934 (g)	Triethanolamine (g)	Disodium EDTA (g)	Propylene glycol (g)	D.M. water (100 g)
F1	0.5	1.5	1.5	0.005	5	Q. S
F2	1	1.5	1.5	0.005	5	Q. S
F3	1.5	1.5	1.5	0.005	5	Q. S
F4	2	1.5	1.5	0.005	5	Q. S
F5	2.5	1.5	1.5	0.005	5	Q. S
F6	3	1.5	1.5	0.005	5	Q. S

Quality control of topical polyherbal gel formulation
Estimation of active constituents in polyherbal gel formulation (net content)
Each formulation (1 g) was added to a 50 mL volumetric flask, which was then filled with methanol to dissolve the active components. The solution was filtered through a Whatman filter paper, and 0.1 mL of the filtrate was pipetted out and diluted with methanol to make a volume of 10 mL. The number of active elements was ascertained spectrophotometrically using a standard curve created at a certain wavelength (max of the active compounds in the extracts).

Extrudability
A closed collapsible tube containing 20 g of nanogel was then tightly clamped to stop any rollback. After removing the cap, the nanogel was extruded. The collected extruded nanogel was then weighed. The amount of nanogel that was extruded was measured.

pH measurement
The pH of the nanogel was determined using a digital pH metre by completely encasing the glass electrode in the nanogel system. Three measurements were made, and the average of the results was noted.

Appearance and Homogeneity
Visual perception was used to assess the generated nanogels' physical characteristics and uniformity.

Viscosity
Using a Brookfield viscometer (S-62, model LVDV-E) set to 25 oC and rotating at 12 rpm, the viscometer's spindle speed was used to measure the viscosity of nanogel.

Spreadability
From two sets, glass slides with consistent diameters were chosen. The herbal nanogel formulation was applied to one of the slides. The other slide was placed on top of the nanogel, which was sandwiched between the two slides in a region that measured 7.5 cm along the slides. 100 g of nanogel was spread uniformly over the top slides before being sandwiched between the two slides to create a thin layer. Once the weight was taken off, extra nanogel that had been adhering to the slides was scraped off. The two slides were fixed in place such that only the upper slides could be freed by the weight being connected to it and there was no least movement. A 20 g weight was carefully fastened to the upper slide. The upper slide moved 7.5 cm in the time required to separate from the lower slide under the weight's impact. The experiment was carried out three times, with the mean time being used to compute the outcomes each time. Using the following formula, spreadability was calculated:
S= m × l/t
where, S= spreadability, m-weight tied to upper slides (20 g), l- length of the glass slide (7.5 cm), t- time taken in sec.

In vitro permeation in rat skin
All formulations underwent in vitro diffusion experiments using Franz diffusion cells. The 3.7994 cm^2 area and 3.8 cm^2 diffusion area of the locally produced open-ended cylindrical tube that makes up the diffusion cell equipment. Phosphate buffer (pH 7.4) was used as the receptor media. Rat abdomen skin was used as a dialysis membrane. Due to the skin's adhesion to the donor cell's diffusion cell, the stratum corneum side of the skin was in close contact with the formulation's release surface. Before being mounted on the diffusion cell, a donor compartment received 100 mL of an isotonic phosphate buffer solution with a pH of 7.4. Weighed one gramme of nanogel-equivalent formulation was placed to the skin of the rat and was continuously swirling in 100 mL of receptor media. The overall system was maintained at 37°C. A 5 mL sample was obtained at specified intervals up to 8 hours, and its concentration was evaluated spectrophotometrically at a predetermined wavelength. Equal portions of the diffusion medium were removed, and then fresh diffusion medium was added. The cumulative % release for each time interval (in h) was calculated.

Release kinetics
To ascertain the release pattern of the active ingredient from the herbal nanogel, data were gathered and fitted to various mathematical models. While zero order kinetics is a concentration independent kinetics, first order kinetics is a dependent kinetics in which drug release may happen after swelling and erosion or only diffusion. The data were verified and the result was established using Higuchi's model.

Stability studies of topical polyherbal nanogel formulation
The main objective of stability testing is to provide evidence of how the quality of the drug product changes over time under the influence of temperature and humidity. The topical herbal nanogel formulation's stability examination took place over a 6-month period in a stability chamber in accordance with ICH guidelines. In a humidity chamber (Floor standing model, 3 units in one with individual humidity and temperature controller, 300 X 300 X 300 mm, 15- 60°C, Technico, India) at 25°C 2°C/60% RH 5% RH, 32°C 2°C/60% RH 5% RH, and 40°C 2°C/75% RH 5% RH, the chosen topical polyherbal nanogel formulation including at the first, first, second, third, and sixth months, samples were taken out and tested for sterility and changes in colour, odour, homogeneity, pH, viscosity, net content, and microbial load.

Anti-arthritic activity
The efficacy of the topical polyherbal nanogel formulation was evaluated using a rat arthritic model caused by FCA. The complete population of rats was divided into four groups, each consisting of six rats. Group 1 received topically applied doses of polyherbal nanogel base as the standard control. In groups 2 to 4, arthritis was brought on by injecting a 0.1 mL (0.1% w/v) suspension of dead Mycobacterium tuberculosis bacteria homogenised in liquid paraffin into the left hind foot's sub plantar region. The arthritic control group was Group 2. In Groups 2 to 4 that had received FCA, arthritis

was allowed to develop for a total of 21 days. On days 4, 8, 14, and 21 of the experiment, rat paw volume and body weight from the control and treatment groups were measured using a digital Vernier calliper. The left knee joint region of Group 3 (used as the reference standard) and Groups 4 were treated topically for 22 to 42 days with diclofenac sodium gel (Voveran gel, purchased from a neighbourhood pharmacy) and the polyherbal nanogel formulation F4, respectively, after the onset of arthritis was confirmed. Using a digital Vernier calliper, the volume of the rats' paws in the control and treatment groups as well as their body weight were measured on the 25th, 29th, 35th, and 42nd days of the treatment period. The outcomes of the pain test on the animals were noted at the conclusion of the 42nd day.

Result and Discussion

Selection and Standardization of Plant Material and Excipients

Leaves of *Vitex negundo*, *Ehretia laevis* and rhizomes of *Curcuma amada* fresh part were gathered and verified. Sigma-Aldrich was used to acquire Freund's complete adjuvant (FCA), diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate. We bought carbopol 934 and carbopol 940 from Mumbai's Loba Chemie Pvt. Ltd.

Preparation of extracts

After carefully removing any remaining materials and earthy residue from the Leaves of *Vitex negundo*, *Ehretia laevis* and rhizomes of *Curcuma amada* they were cleaned and allowed to dry in the shade. Using a 7-day cold maceration method, methanol was extracted from coarsely powdered of all three herbs. The extracts were then kept at 4-8 o C for later use after being filtered and concentrated under decreased pressure in an IKA Rotary evaporator (Model No RN 10 digital V, ILMAC Germany).

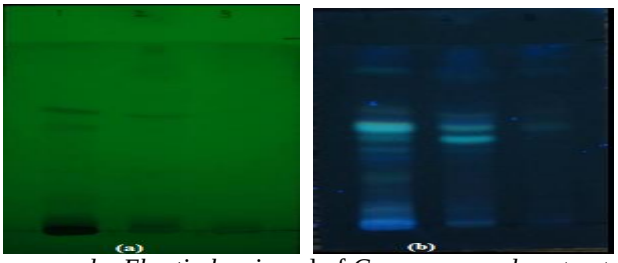


Figure: TLC profiles of *Vitex negundo*, *Ehretia laevis* and of *Curcuma amada* extracts at (a) 254nm and (b) 366nm

Phytochemical analysis of polyherbal extract

Using the solvents methanol, ethanol, petroleum ether, and chloroform, the phytochemistry of polyherbal extract was investigated. The methanol extract contains all the ingredients from all three herbs, including sugars, alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds, and steroids. However, the polyherbal ethanol extract contains all ingredients other than carbohydrates. Steroids, alkaloids, flavonoids, tannin, terpenoids, glycosides, phenolic compounds, and tannins are among the chemical compounds. The chloroform extract of polyherbs contains steroid, glycosides, alkaloids, flavonoids, terpenoids, and carbohydrates. Both tannin and phenolic compounds are absent. The petroleum ether extract of polyherbs contains sugars, tannin, flavonoids, steroids, and other chemical compounds. The major components in a blend of all three herb preparations include flavonoids, terpenoids, and steroids. The table below presented the findings.

Table: 1 Preliminary Phytochemical screening of various extracts of the mixture of all three herbs (Vitex negundo's, Khanduchakka and Ambehadad)

Constituents	Ethanol	Methanol	Petroleum ether	Chloroform
Alkaloids	-	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Carbohydrate	-	+	-	+
Terpenoids	+	+	+	+
Glycosides	+	+	-	+
Steroids	+	+	+	+
Phenols	+	+	-	-

Where,
+ Present
- Absent

Green Synthesis of AgNPs

AgNPs were created in a sustainable manner by using plant extract. The colour of the silver nitrate solution changed when plant extract was added, going from light yellow to dark brown, signifying that silver ions had been reduced and silver nanoparticles had produced.

Characterization of Silver Nanoparticles

UV-Visible Spectral Analysis

In their UV absorption spectra, all three polyherbal extracts show a unique peak in the range of 400 to 450 nm. The peak specificity in this region can be caused by the Mie scattering phenomena. The UV spectrum of the silver nanoparticle is shown in Figure 1.

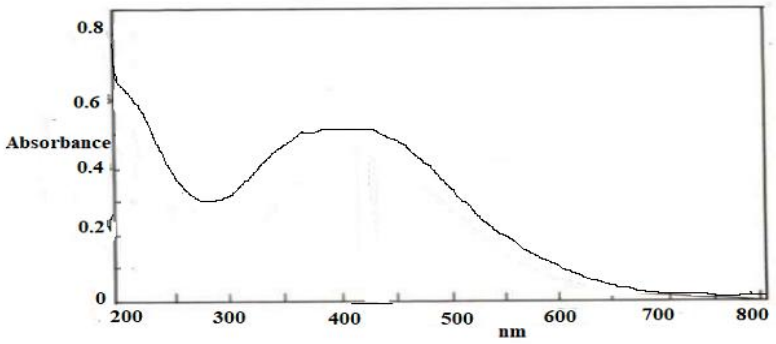


Fig. 1: UV-Visible spectra of Silver Nanoparticles

Using a zeta sizer in dynamic light scattering mode, the average particle size in the aqueous reaction mixture was determined after the reaction was complete. The average particle size of 201 nm revealed that the silver ions were transformed into nanoparticles. The polydispersity index (PDI), which gauges homogeneity and globule distribution, was found to be 0.376 for silver nanoparticles. Particle size and polydispersity index are shown in Figure 2.

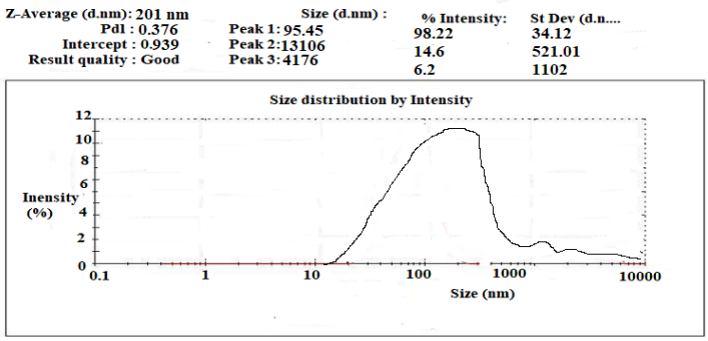


Fig. 2: Particle size and poly dispersity index of silver nanoparticles

FT-IR Spectroscopy Characterization

According to the FTIR spectra of Ag nanoparticles (Fig. 3b), phenols and alcohols exhibit O-H stretching vibrations in the band at 3407.31 cm^{-1}. Stretch alkanes (C-H) connected to a pronounced band. The peaks at 2946.32 cm^{-1}, 1591.30 cm^{-1}, 1382.02 cm^{-1}, and 739.71 cm^{-1}, corresponding to the C-H stretch alkanes, carboxylated group, asymmetric stretching vibrations of methylene groups, and alkenes group, were each used to represent the corresponding group. This indicated that phytochemicals from the extracts of all three herbs were present near the Ag nanoparticles that were generated. The polyherbal extract's FTIR spectra showed a peak at 1242.16 cm^{-1} that corresponded to the C-O stretch of alcohols (Fig. 3a). The N-H stretching of an aromatic secondary amine and the C-N stretching of aromatic amine groups, respectively, were indicated by the peaks at 1541.12 cm^{-1} and 1348.24 cm^{-1}. These functional groups are represented by the flavonoids, phenols, alkaloids, saponins, and carbohydrates present in the aqueous extracts of all three herbs. Earlier phytochemical analyses on the leaf extract had revealed their presence.

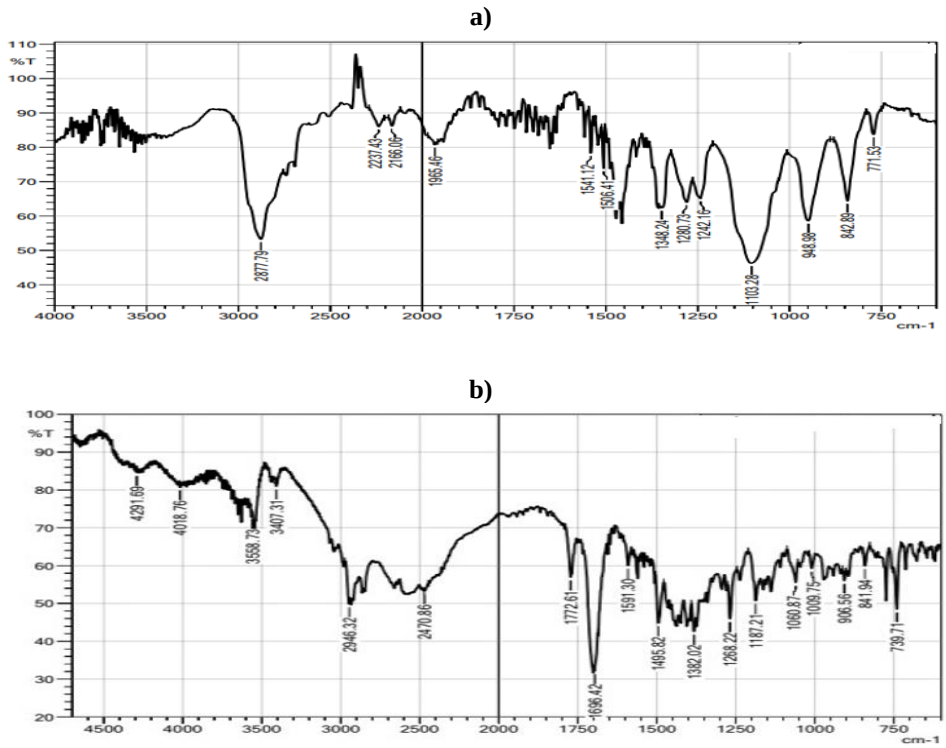


Fig. 3: a) FTIR spectrum of polyherbal extract. **b** FTIR spectrum of Ag nanoparticles.

Entrapment efficiency (%)

The success of entrapping silver nanoparticles. After that, entrapment's effectiveness did not really increase. The efficiency of entrapment was found to be 82.78%. The best efficiency % was determined using polymers.

XRD Analysis of Silver Nanoparticles

Based upon the characteristic peak obtained (20 value taken from the graph Figure), the particle size of the crystal was calculated using the formula,

$$D = {}^{0.9\lambda}/_{\beta\cos\theta}$$

where, D represents the average size of the particle, λ corresponds to wave length of copper Ka line (1.5406 A°), ß represents full width at half maximum of peak, and corresponds to diffraction angle. The size of the particle (D) was calculated as 5.3 nm.

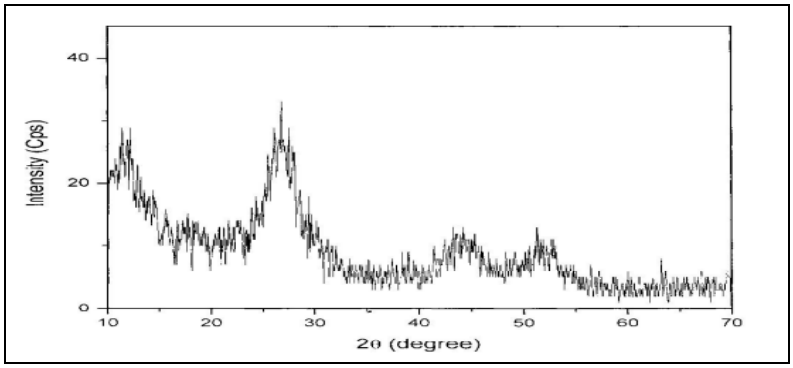


Figure: XRD pattern of silver nanoparticles

Scanning Electron Microscopy (SEM) Analysis
The SEM image represents the formation of silver nanoparticles. It was observed in Figure. the grains were aggregated to form nanoclusters.

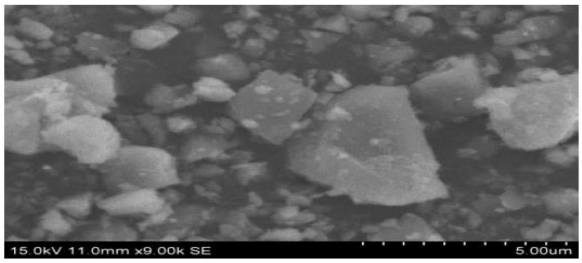


Figure: SEM image of silver nanoparticles

Transmission electron microscopy
The size and shape of the S1 are shown in Figure 4. They were investigated using transmission electron microscopy (TEM). The particle sizes and shapes were measured using various fields. The particles in the electro-silver micrograph were round and had distinct particle sizes. The size has been reported as the mean diameter in figure 4. The spherical form and strictly controlled particle sizes of the silver nanoparticles are visible. Furthermore, as might be expected, preparation conditions have a big impact on particle size.

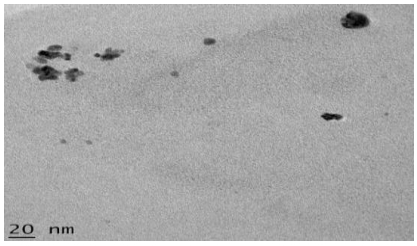


Figure 4. TEM images of spherical silver nanoparticles, the scale bars were 50 nm and magnifications 50 kx.

In-vitro dissolution study
There was absolutely no drug release in simulated stomach juice (pH 1.2 acidic) for the first 90 minutes, according to trials with silver nanoparticles in vitro. Drug release (pH 6.8 phosphate buffer) was present in both the colonic medium and the simulated intestinal fluid (pH 7.4 phosphate buffer). In-vitro release profiles'-controlled efficacy in intestinal or colonic media was shown to be high. The kind of polymer matrix and the medium pH were both found to have an impact on the drug release during the dissolving research. Across all formulations, higher polymer content led to noticeably higher drug release rates. The silver nanoparticles showed a larger drug release during a 90-minute period. The percentage cumulative drug release of silver nanoparticles in pH 7.4 phosphate buffer was found to be 89.23% after 90 minutes. Based on the findings of in-vitro drug release studies, silver nanoparticles have shown favourable drug release rates.

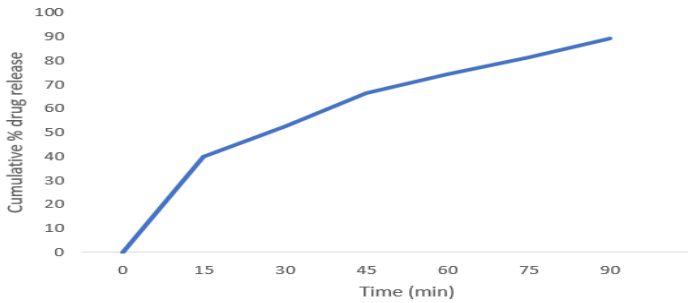


Fig.5: In- vitro dissolution profiles of all three herbs extract loaded silver nanoparticles at Phosphate Buffer Solution pH 7.4

Quality control test for formulated silver nanoparticle encapsulated polyherbal nanogel

Twelve carbopol nanogel formulations, F1 to F6, had their physical characteristics, pH, viscosity, spreadability, net content, extrudability, and in-vitro diffusion profile evaluated. Table 2 lists the specifics of the study's outcomes, which satisfied ICH requirements.

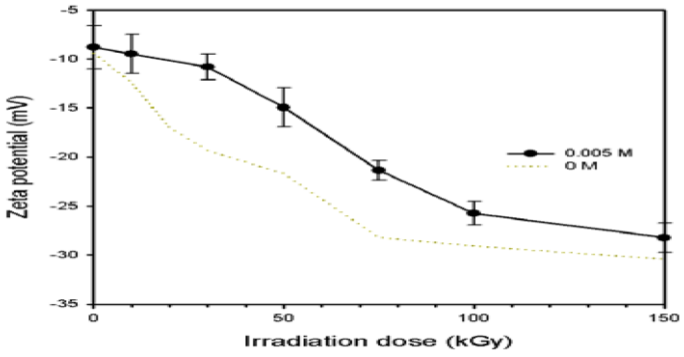
Table 2: Evaluation parameters for topical polyherbal nanogel formulation made with 1.5% Carbopol 934

Code	Conc. (%)	pH	Viscosity (poise)	Spreadability (g cm/sec)	Drug content (% w/w)	Extrudability	Physical appearance
F1	0.5	7.45	0.3973	34.45	97.12	Good	Greenish, smooth and translucent
F2	1.0	7.58	0.3954	46.21	99.45	Excellent	Dark green, smooth, homogenous, translucent
F3	1.5	7.75	0.4064	53.12	98.34	Good	Dark green, smooth, homogenous, translucent
F4	2.0	7.58	0.4187	61.34	102	Excellent	Dark green, smooth, homogenous, translucent
F5	2.5	7.87	0.4222	75.99	99.83	Excellent	Dark green, smooth, homogenous, translucent
F6	3.0	7.46	0.4298	77.12	104	Excellent	Dark green, smooth, homogenous, translucent

The prepared nanogels were found to be homogenous, acceptable, and consistent. All the formulations' pH values fell within the constrained range of neutral pH (7.45-7.87), according to a study on skin sensitivity. There was no skin irritation as a result. Polymers were added to the developed topical formulations to provide a quick release of the medicine to reach and maintain the medication concentration within the therapeutically suitable range. Since the polymer concentration was set at 1.5% in each of the polyherbal nanogel formulations, no variations in viscosity were observed. Additionally, it was claimed that a topical nanogel formulation created with carbopol polymers had a best viscosity value between 0.39 and 0.42 poise. Spreadability numbers demonstrated how easy it is to spread the nanogel compositions. All of the nanogel formulations from F1 to F6 had exceptional extrudability scores (>90% extrudability, >80% extrudability, >70% extrudability, fair), with the exception of F1 and F3, which had 90% of the contents that could be extruded.

Zeta Potential

The net surface charge of a nanoparticle when it is submerged in a solution is known as a zeta potential. A significant negative or positive zeta potential determines whether particles will push against one another and how they will aggregate. The stability of solutions is constrained by the zeta potential, which is significant. It can be either +30 mV or 30 mV. Ag/PAAc nanogels' zeta potential as a function of radiation dosage are depicted in Figure. Figure illustrates how increasing irradiation doses raised the zeta potential's absolute value. The Ag/PAAc nanogels' maximum zeta potential was around 27 mV at 150 kGy. As a result, the Ag/PAAc nanogels at 150 kGy were evenly dispersed and unable to aggregate**.**



***In vitro* diffusion profile and release kinetics**

The in vitro diffusion profile for the F1 through F6 formulations is shown in Figure 6. Because the pH of the membrane in use fluctuated from 5 to 7.8, phosphate buffer saline pH 7.4 was used for the in vitro release studies of the nanogel formulations. According to their in vitro release characteristics, all six formulations containing carbopol 934 caused a virtually 100% release from the formulation within 5 hours. The in vitro release characteristics of the created topical polyherbal silver nanoparticles loaded nanogel formulations were very encouraging and consistent with commercial diclofenac gel. F4 demonstrated superior release characteristics (97.5%) compared to the other formulations (Figures 7 to 9) than did F1, F2, F3, F5, and F6. We concluded that the F2 formulation corresponded to zero order kinetics after conducting a kinetic release analysis. Since zero order kinetics is preferred for sustained release, a nanogel formulation containing 2% extract of all three herbs silver nanoparticles was chosen for in vivo tests. Commercial diclofenac sodium gel formulation released 90% of its substance in 3 hours, whereas F2, which contains 2% of the extracts of all three

herbs, prolonged the release of active ingredients up to 5 hours (almost 100%), making it ideal for continuous release and enhancing patient compliance. The nanogel formulation containing 2% extract of each of the three herbs listed in Table 3 was therefore shown to have zero order release kinetics according to the release data acquired using various mathematical models. Since zero order kinetics follows controlled release, the nanogel formulation F2, which contains 2% of extract, was chosen for in vivo experiments.

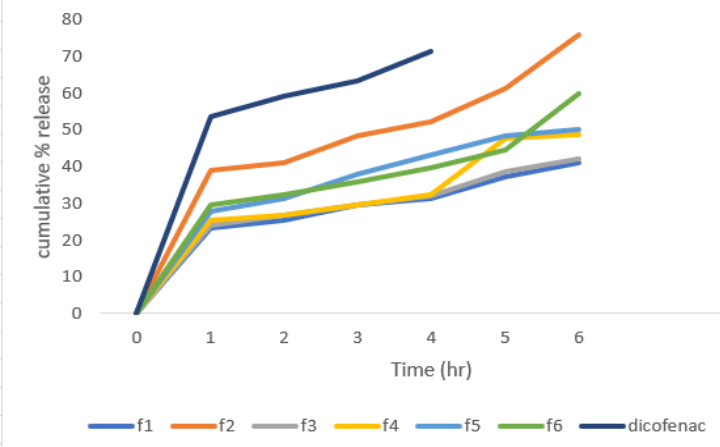


Fig. 6- *In vitro* diffusion profile of topical polyherbal nanogels (F1-F6) and diclofenac sodium gel.

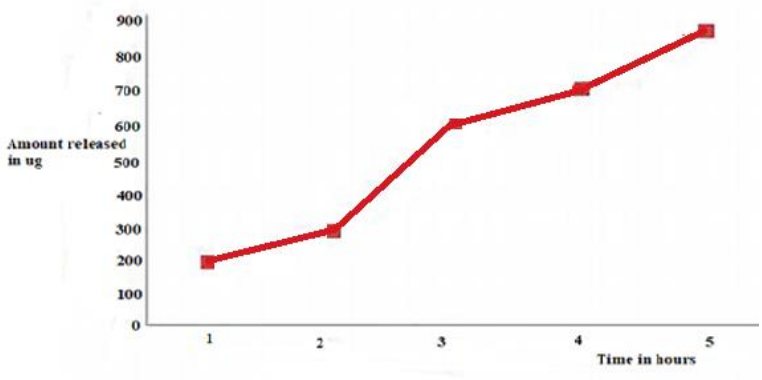


Fig. 7 - Zero order plot for F2 topical polyherbal nanogel formulation.

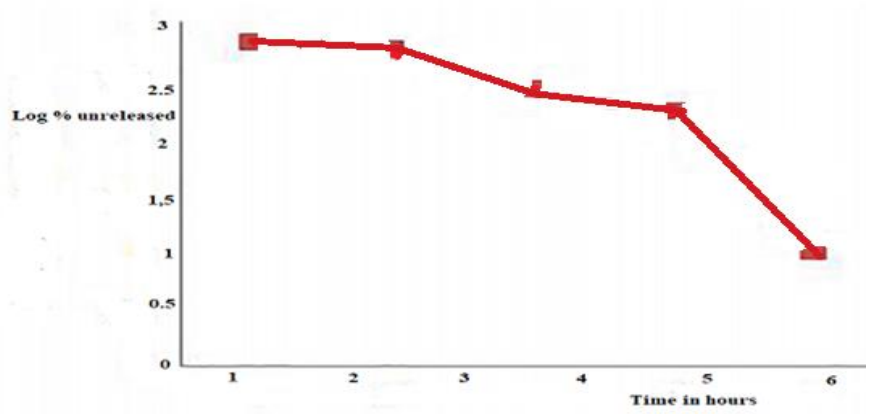


Fig. 8: First order plot for F2 topical polyherbal nanogel formulation.

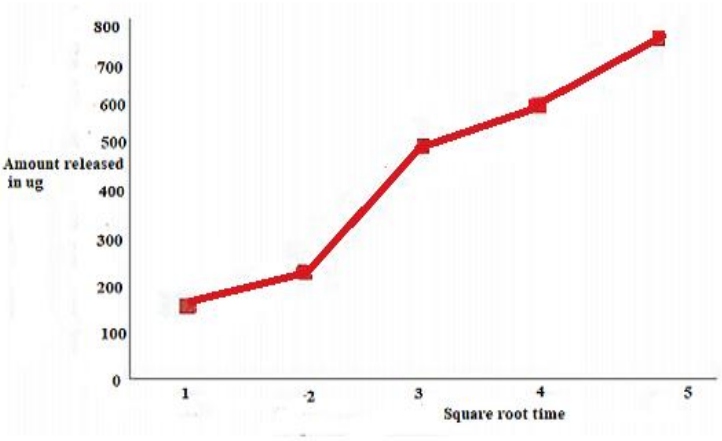


Fig. 9- Higuchi diffusion plot for F2 topical polyherbal nanogel formulation.

Table 3 - *In vitro* release kinetic study of topical polyherbal nanogel formulated with Carbopol 934

Formulation code	Zero order R^2	First order R^2	Higuchi diffusion model R^2	Best fitted model
F1	0.964	0.927	>1	Zero order
F2	0.956	0.945	0.941	Zero order
F3	0.919	0.939	>1	First order
F4	0.987	0.917	>1	Zero order
F5	0.983	0.905	0.909	Higuchi
F6	0.923	0.889	0.921	Higuchi

Skin irritation test

Indicating that the created polyherbal nanogel formulation was determined to be safe when the skin irritating impact of the polyherbal nanogel was examined, no erythema or edoema was observed for any of the formulations, even after 10 days of inquiry (Table 4).

Table 4: Primary skin irritation test for polyherbal nanogel formulation 934

	Rabbit Numbers					Combined index
	Rabbit Number			Rabbit		
	1	2	3	Control	Average	
1 h						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0	0.00	
24 h						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0	0.00	
48 h						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0	0.00	
72 h						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0	0.00	
7 days						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0	0	0.00
10 days						
Erythema score	0	0	0	0	0.00	0.00
Edema score	0	0	0	0.00		

Stability testing

Since the F2 formulation (created using carbopol 934) demonstrated improved quality attributes, stability studies were conducted in line with ICH criteria to guarantee the quality, safety, and efficacy over the course of the shelf life. The topical polyherbal gel formulation's colour, aroma, homogeneity, pH, viscosity, or net content did not alter during stability tests for 0, 1, 2, 3, and 6 months. The topical nanogel F2 formulation's stability was clearly demonstrated by the study's results (Table 5).

Table 5: Stability studies of topical polyherbal nanogel formulation

Sr. no.	Parameters	Topical polyherbal nanogel formulation (F2) containing 2% w/v of all three herbs extract														
		Storage condition														
		25 °C ± 2 °C/60% RH ± 5% RH					32 °C ± 2 °C/60% RH ± 5% RH					40 °C ± 2 °C/75% RH ± 5% RH				
		Months					Months					Months				
		0	1	2	3	6	0	1	2	3	6	0	1	2	3	6
1	Colour	No change in Colour					No change in Colour					No change in Colour				
2	Odour	No change in odour					No change in odour					No change in odour				
3	Homogeneity	Smooth					Smooth					Smooth				
4	pH	6.38	6.37	6.31	6.29	6.26	6.35	6.32	6.34	6.27	6.24	6.34	6.31	6.26	6.24	6.22
5	Viscosity (poise)	0.381	0.378	0.372	0.367	0.362	0.373	0.371	0.372	0.368	0.365	0.379	0.372	0.366	0.361	0.352
6	Drug content (%)	99	99	98	95	95	97	96	96	95	96	97	95	94	93	93
7	Microbial load (Bacteria and fungi)	No microbial growth was observed at 24,48 and 72 h					No microbial growth was observed at 24,48 and 72 h					No microbial growth was observed at 24,48 and 72 h				
8	Sterility test	No microbial growth was observed at 24,48 and 72 h					No microbial growth was observed at 24,48 and 72 h					No microbial growth was observed at 24,48 and 72 h				

Body weight

The average reduction in body weight was seen across all groups after the introduction of rat arthritis (Table 6). The diclofenac sodium gel and topical polyherbal nanogel formulation F2 treated groups showed increases in body weight compared to the normal group of rats, whereas the arthritic control group displayed weight loss.

Table 6: Effect of diclofenac sodium, F2 polyherbal nanogel formulation on body weight changes in FCA Induced arthritic rats

Groups	Initial body weight (g)	Body weight after 21 days of FCA induction	Body wt after treatment 25 th day	Body wt after treatment 29 th day	Body wt after treatment 35 th day	Body wt after treatment 42 th day	Weight gain (g)
Normal control	146.3 ± 0.65	163.4 ± 1.81	164.2 ± 1.86	168.34 ± 1.34	176.34 ± 1.23	183.56 ± 1.33	25.46 ± 1.78
Arthritic control	136.4 ± 0.78	147.48 ± 0.83	132.11 ± 0.67	129.41 ± 1.23	138.00 ± 1.65	123.76 ± 1.45	-14.00 ± 1.67
Diclofenac sodium topical gel (1% w/w)	136.00 ± 1.26	139.65 ± 1.34	149.00 ± 1.34	123.21 ± 1.45	137.65 ± 1.76	155.65 ± 1.76	7.56 ± 0.87
Topical polyherbal nanogel formulation (2% w/w)	156.6 ±1.07	148.65 ± 1.23	146.21 ±1.46	134.00 ±1.54	148.82 ± 1.60	138.51 ± 1.68	6.45 ± 0.87

Paw volume

Changes in rat paw volume were observed on days 25, 29, 35, and 42 after topical application of diclofenac sodium gel and the polyherbal nanogel formulation (F2) for 22 to 42 days (Table 7 and Figure 10). The development of arthritis was detected by the rise in paw volume in the arthritis control groups. Rat paw volume was significantly ($p<0.01$) reduced in groups given topical polyherbal nanogel formulation F2 and diclofenac sodium gel on the 21st day after FCA induction. Methods for assessing the severity of arthritis visually were employed. The arthritic test results, which are described in Table 8, revealed that FCA-induced arthritis caused much reduced discomfort in the groups treated with topical polyherbal nanogel formulation F2 and diclofenac sodium gel. The flexion pain test score, mobility score, and stance score for each treated group of rats significantly changed as compared to the arthritic control group of rats. This modification in the outcomes of the arthritic tests supports the anti-arthritic effects of the topical polyherbal nanogel formulation F2. Out of formulations F1 to F6, formulation F2 was selected for the anti-arthritic study because its quality control evaluation results were favourable and it's in vitro release characteristics were favourable and consistent with those of diclofenac sodium gel that is sold commercially. FCA-induced arthritis is the most well-liked model with clinical and pathological abnormalities like those reported in human rheumatoid arthritis. It is unusual for rats treated with FCA to develop polyarthritis, and this condition is linked to an immune-mediated inflammatory response. The selective reduction of the arthritic score (Table 8) and the significant weight reduction of the thymus and spleen in all treated groups when compared to the arthritic rats provided evidence for the anti-arthritic activity.

Table 7: Evaluation of anti-arthritic activity of polyherbal nanogel formulation F2 in FCA induced arthritic rats

Groups	Rat paw volume (mm)					
	Before treatment	After treatment				
	Initial	After 21 days	25th day	29th day	35th day	42nd day
Normal control	3.76 ± 0.68	4.45 ± 0.34	5.34 ± 0.56	5.45 ± 0.21	5.75 ±0.23	5.65±0.84
Arthritic control	5.23 ± 0.23	12.33 ± 0.10	11.57 ± 0.32	11.04 ±0.21	11.78±0.45	12.83±0.13
Diclofenac sodium topical gel (1 % w/w)	5.53 ± 0.34	11.34±0.21	11.42±0.24	9.97± 0.67	9.67±0.47	9.54± 0.08
Topical herbal nanogel formulation F2 (2% w/w)	5.12 ± 0.14	11.13 ±0.22	12.03 ± 0.22	10.01 ± 0.23	9.34 ±0.33	8.98 ± 0.27

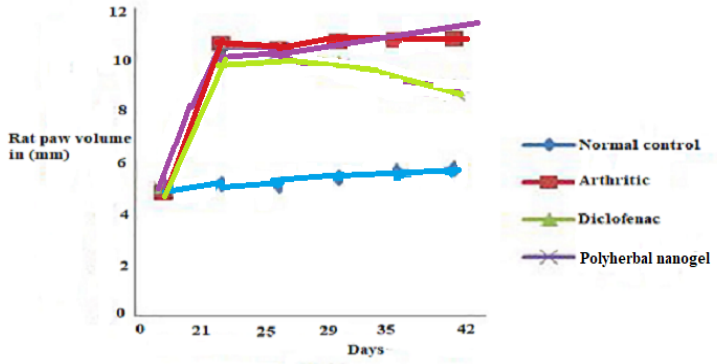


Fig. 10: Polyherbal gel formulation of F2 in FCA induced arthritic rats.

Table 8: Alterations in various pain test scores in FCA Induced arthritis in rats

Groups	Pain test		Mobility score	Stance score
	Extension	Flexion		
Arthritic control	8.9 ± 0.96	9.04 ± 0.23	2.22 ± 0.14	2.75 ± 0.12
Diclofenac sodium topical gel (1% w/w)	6.08 ± 0.06	5.21 ± 0.42	3.80 ± 0.34	3.45 ± 0.12
Topical polyherbal nanogel formulation (2% w/w)	5.74 ± 0.14	4.71 ± 0.12	4.25 ±0.54	3.84 ± 0.33

Conclusion

In the present study, silver nanoparticles (AgNPs) were synthesised from the extracts of all three herbs (Leaves of *Vitex negundo*, *Ehretia laevis* and rhizomes of *Curcuma amada*) in a simple, efficient, and economical manner. The synthetic AgNP made from this polyherbal extract showed greater activity than the extract itself. The silver nanoparticles' nanogel was made using a few gelling agents after the synthesis of the polyherbal extract, and the resulting silver nanoparticles nanogel formulation was analysed. The anti-arthritic activities of the newly discovered topical polyherbal nanogel formulation could be related to luteolin, which is present in methanol extracts of polyherbal silver nanoparticles. The developed formulation F2, which contains 1.5% of carbopol 934 and 2% of the three herbs' extracts, was found to be a promising topical polyherbal nanogel for the treatment of arthritis. The utility of this formulation for persons with joint inflammatory disorders may be supported by more clinical studies.

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