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DEVELOPMENT OF GEL WITH CURCUMIN SILVER NANOPARTICLES AND EVALUATION OF ITS *IN-VIVO* WOUND HEALING AND ANTIMICROBIAL CAPABILITIES

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Keywords:

Curcumin, Silver nanoparticles, Wound healing, Antimicrobial, Excision wound model

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ABSTRACT: Silver nanoparticles (AgNPs) are metallic nanoparticles that play an important role in nanomedicine as antimicrobial agents, wound management, anti-diabetic, burn care, cancer diagnosis and therapy, etc. Natural polyphenol curcumin, which is derived from turmeric, is well-known for its ability to heal wounds and has been shown to have antibacterial, antioxidant, and anti-inflammatory properties. Although curcumin is used more widely, its hydrophobic nature restricts its effectiveness. To increase its activity, curcumin can be combined with AgNPs with the help of a precursor. In the current work, curcumin-AgNP (CUR-AgNP) was made chemically with sodium citrate acting as a reducing agent and silver nitrate serving as a precursor. The prepared AgNP was characterized for particle size, PDI, zeta potential, Fourier-transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), X-ray diffraction (XRD). Carbopol was used to convert the CUR-AgNP into a gel, which was then characterized in terms of pH, gel strength, viscosity, and spreadability. The capacity of the targeted gel formulation to heal wounds *in-vivo* was examined in Albino Wistar rats. The results showed that the prepared gel possessed good physicochemical properties with good stability. Using an excision wound model, the *in-vivo* healing activity was shown to be 96.96%, while the marketed silver nitrate gel (0.2% w/w) demonstrated 95.41% healing. The above results confirmed the effectiveness and enhanced topical delivery of the prepared formulation which could be better than the marketed preparation.

INTRODUCTION: Nanotechnology is a rapidly developing field that is being used in science and technology to create new materials at the nanoscale level [1]. Nanoparticles (NPs) are solid particles having nanometer sized (<100 nm). Depending on their size and form, nanoparticles are solid atomic or molecular scale particles with exceptional physical properties compared to bulk molecules [2].

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A variety of nanomaterials, including those made of gold, silver, titanium, copper, magnesium, zinc, alginate *etc*. have been developed. However, due to the ability to penetrate cell membranes and possible metabolic activity, silver nanoparticles have become the most successful due to their powerful antibacterial characteristics against bacteria, viruses, and other eukaryotic microorganisms [2, 3].

Researchers from numerous academic laboratories have become interested in silver nanoparticles among the metal nanoparticles because of their new chemical, physical, and biological features compared to their bulk form [4]. Some organisms resist silver's antibacterial effects. Silver ions,

released from silver-containing materials, interact with thiol groups in proteins and enzymes, disrupting cell respiration and causing bacterial cell death. However, silver ions lose solubility and antibacterial properties when binding to halide ions like chloride [5]. To overcome this limitation, silver nanoparticles have been developed for enhanced antibacterial activity [6, 7]. In recent years, silver nanoparticles have shown a considerable antibacterial effect to fight against illness and infection [8]. Since ancient times, silver has also been used to treat wounds. Silver-based products and AgNPs based biomedical products, such as wound dressings, are currently commercially available for a variety of medical applications due to their broad-spectrum antibacterial capabilities [9]. This study describes the creation of a gel loaded with curcumin silver nanoparticles. Curcumin (CUR) is a hydrophobic polyphenolic molecule, extracted from the rhizome of *Curcuma longa*, with a range of biological activities, including wound healing and antibacterial, antioxidant, anti-inflammatory, and anti-cancer actions [10]. However, it has an important issue such as its solubility in water and susceptibility to breakdown due to pH changes [11]. To overcome its solubility and bioavailability problems, curcumin-loaded nanoparticles have been reported by numerous researchers. Based on the above background, the current study develops Curcumin silver nanoparticles loaded gel to provide innovative and improved wound healing and antibacterial activity.

MATERIALS AND METHODS: Silver nitrate was obtained from Loba Chemie Pvt. Ltd. Sodium citrate was procured from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India). Curcumin was obtained from Sigma Aldrich. Distilled water was used to prepare the solutions.

Synthesis of Cur-AgNPs: Silver nanoparticle was prepared by dissolving silver nitrate (0.001M) in 50ml distilled water and heating for 5-10mins at 80℃ under continuous stirring. 1 ml of 1% sodium citrate solution was added to the previous mixture and heated for about 15-20minutes at 80℃ under continuous stirring until the colour changed to pale yellow and then the solution was cooled at room temperature [12]. Curcumin was dissolved in DMSO and added to the above solution under continuous stirring for 20- 30minutes. After the colour changed from pale yellow to reddish brown, the solution was stored for later use in a cold, dark area.

Characterization of Cur-AgNPs:

Particle size and Morphology: The particle size and size distribution of the nanoparticles were determined using the particle size analyser (Malvern Zeta Sizer). The Zeta Sizer (Nano ZS90, Malvern Analytical) was used to measure the surface zeta potentials. Transmission electron microscopy (TEM) was used with a TEM-2100 PLUS electron microscope to examine the morphology of the produced nanoparticles [13].

X-Ray Diffraction Study (XRD): A powder sample of CUR-AgNP was employed to determine the X-ray diffraction (XRD) pattern by using the Phillips X'Pert Pro Powder X-ray diffractometer run at 45 kV with a current of 40 mA utilizing Cu-Kα/radiation (λ = 1.54060 Å). The diffracted intensities were measured and recorded from 4° to 89° at 2θ angles.

***In-vitro* Drug Release Study:** The *in-vitro* release of CUR-AgNPs was performed by using dialysis membrane using phosphate buffer (pH 7.4) with 1% Tween 80 at 37℃. Samples were examined at a wavelength of 420 nm using a UV Spectrophotometer [14].

Preparation of Plain Gel: Carbopol 940 was dissolved in demineralized water with constant stirring until it was entirely dissolved. Triethanolamine was added dropwise while being constantly stirred to produce a transparent gel. The prepared gel was then evaluated for physicochemical parameters as mentioned in the evaluation part [15].

Evaluation of the Carbopol Gel:

pH and Viscosity of the Gel: The pH of the gel was measured by using a digital pH meter (SYSTRONICS). Using a Brookfield viscometer (S-64, model BROOKFIELD, LVDV-E) at 25°C and a spindle speed rotation of 1–20 rpm, the viscosity of the produced gel was determined [15].

Spreadability: The gel formulation was sandwiched between two slides, spaced 7 cm apart. The upper slide, attached to an 18 g weight, was released, and the time taken to detach was recorded. This process was repeated three times,

and the mean value was calculated to assess gel adhesion properties [16]. The following formula was used to determine the spreadability value:

$$S = m \times l/t$$

(where, S is spreadability, m is the weight tied to upper slides (18 g), l is the length of the glass slide (7cm), and t is the time taken in sec.

Gel Strength: A texture profile analyzer (Stable Micro System, FD/1-077) was used to measure the developed gel's strength.

Loading of AgNPs in Gel: After optimization of the Plain carbopol gel, 60 mg of the manufactured AgNPs formulation was added, and for 30 minutes, the gel was continuously stirred mechanically to ensure that the AgNPs were distributed uniformly. Then the gel was characterised by pH, viscosity, spreadability, and gel strength.

***In-vitro* Antimicrobial Activity:** The antimicrobial property of the prepared curcumin-loaded silver nanogel was verified by Kirby Bauer disc diffusion method against the standard test microorganisms. Fresh bacterial culture strain *Staphylococcus aureus* was used for the antimicrobial test. Agar plates were prepared with a specific number of bacterial colonies, and the treatments were given in the prepared discs on agar plates and incubated. Following incubation, the zone diameters were measured to the nearest millimeter using a calliper or ruler. Antimicrobial effectiveness of the prepared silver nanogel was determined by measuring the zone of inhibition diameter [17].

***In-vivo* Wound Healing Property:** The wound-healing efficacy of the prepared silver nanogel was assessed using an excision wound model in Wistar albino rats (180–250 g, both sexes). The Institutional Ethics Committee of Girijananda Chowdhury Institute of Pharmaceutical Science approved the study (M.PH/PRO/33/2021). Rats were divided into five groups (six per group): Group I (no wound), Group II (negative control, untreated), Group III (negative control, plain gel), Group IV (positive control, silver nitrate), and Group V (treated with 0.2% w/w formulation). Following CPCSEA guidelines, rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine before creating a 2×2 cm^2 full-thickness excision wound. Treatments were applied daily until complete healing, with wound measurements taken on days 0, 7, 14, and 21 to calculate percentage reduction. After the experiment, all rats were anesthetized, and healed tissue samples were collected for histological analysis [18]. Wound area was measured on days 0, 7, 14, and 21 for each group by tracing the incision on a translucent sheet over millimetre based graph paper. Wound contraction was assessed every seven days until complete healing. The percentage of wound contraction was calculated, considering the initial wound size as 100%.

Wound contraction (%) = (Area of wound on day '0'- Area of wound on day 'n') / Area of wound on day '0'× 100 [19]

The epithelialization period was determined by counting the number of days needed for the wound's dead tissue remains to slip off and leave no trace of the original raw lesion [20].

Histopathological Analysis: All of the animals were given ketamine anaesthesia at the conclusion of the trial, and samples of wound tissue were collected and stored in glass vials with 10% formalin solution for histological analysis. Hematoxylin and eosin (H&E) dye was used to produce and stain sections of wound tissue specimens for histological analysis [21].

Statistical Analysis: The mean±SEM is used to express the results. Two-way analysis of variance was used to compare the results between treatments (ANOVA). A P-value <0.05 is considered statistically significant. Graph Pad Prism 10 was used for all data processing.

RESULT AND DISCUSSION:

Formulation of Silver Nanoparticles: Silver nanoparticles (AgNPs) were synthesized using sodium citrate as a reducing agent and silver nitrate as a precursor. Curcumin, dissolved in DMSO, was added to the silver nitrate solution. Initially, the reaction mixture turned light yellow, indicating nanoparticle formation, and upon curcumin addition, the colour changed to deep brown **Fig. 1**. Various drug-to-silver nitrate ratios were tested, and the optimal formulation was selected based on particle size and polydispersity index (PDI). The nano formulation with the smallest particle size and lowest PDI was chosen for further study, ensuring stability and effective drug delivery.

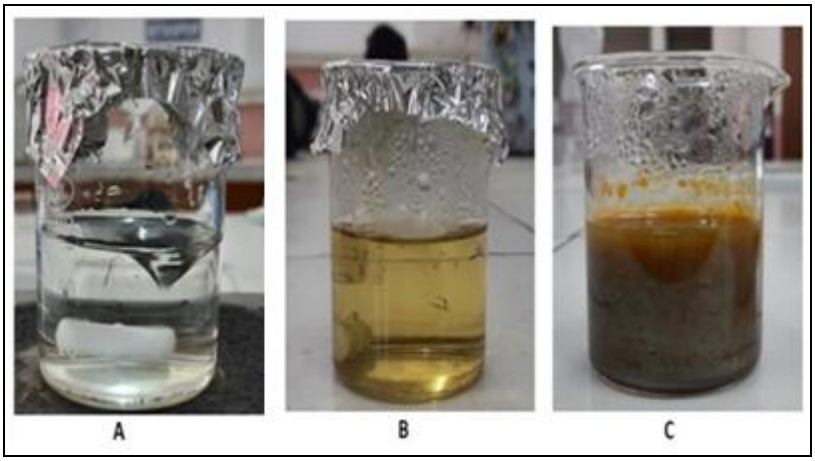

FIG. 1: FORMULATION OF CUR-AgNPs (CHANGE IN COLOUR AFTER ADDITION OF CURCUMIN). A) SILVER NITRATE SOLUTION, B) AFTER ADDITION OF SODIUM CITRATE, C) AFTER ADDITION OF CURCUMIN

Characterization of the Cur-AgNP Formulations:

Particle size and Morphology Analysis: Cur-AgNPs showed particle size from 70 to 103 nm range with PDI 0.31±0.1, indicating the homogeneity of the formulation **Fig. 2A**. Zeta potential value was found negative in the range of -42.9Mv to -40.5Mv which reveals the stability of nanoparticles against aggregation **Fig. 2B**. From the TEM study of the optimized Cur-AgNPs, the particles were found homogeneous and spherical in nature as depicted in **Fig. 2C.**

X-Ray Diffraction (XRD) Analysis: The XRD pattern of Cur-AgNP revealed that the particles were crystalline in nature. As shown in **Fig. 2D**, the significant peaks 38º, 44º, 64.5º, and 77.5º were indexed as (111), (200), (220), and (311). The acquired XRD pattern was examined and matched with the silver file number 04-0783 from the Joint Committee on Powder Diffraction Standards (JCPDS). The XRD pattern of the synthesized Cur-AgNPs is shown in **Fig. 2D.**

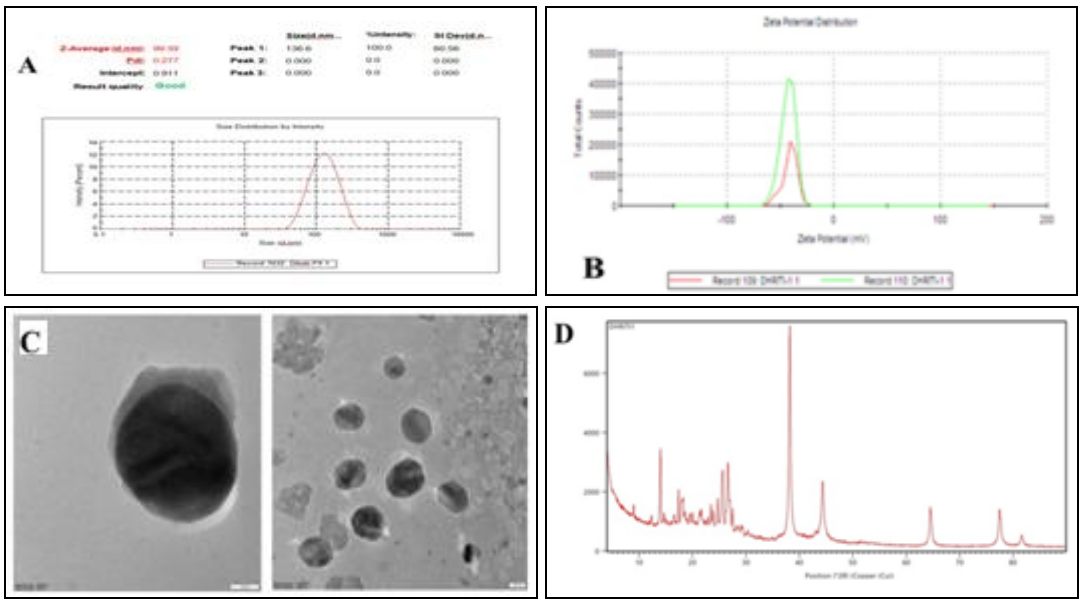

FIG. 2: CHARACTERIZATION OF PREPARED CUR- AgNPs. A. SIZE DISTRIBUTION BY INTENSITY OF THE OPTIMIZED CUR- AgNPs, B. ZETA POTENTIAL ANALYSIS OF CUR-AgNPs, C. TEM MICROGRAPH OF THE CUR- AgNPs, D. XRD ANALYSIS OF CUR- AgNPs

***In-vitro* Drug Release:** Curcumin's release from Cur-AgNPs proved the AgNPs' ability to release content continuously. The findings proved that the release of curcumin from the Cur-AgNPs system was noticeably prolonged in time. In 24 hrs, Cur-AgNPs were able to release 81.27% of the medication. The drug release pattern is shown in **Fig. 3.**

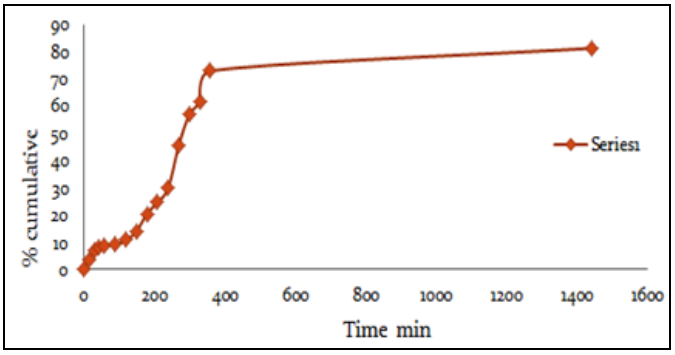

FIG. 3: *IN-VITRO* DRUG RELEASE PATTERN OF CUR-AgNPs

Preparation and Optimization of Plain Gel: Carbopol gel of different concentrations (0.2%, 0.3%, 0.4% and 0.5%) was prepared **Fig. 4A** and evaluated for physical appearance, pH, viscosity, gel strength, and spreadability as shown in **Table 1**.

From the study, no phase separation was observed in any of the prepared gels. Additionally, it was noted that while the formulations' viscosity increased with an increase in the concentration of the gelling ingredient Carbopol, their spreadability and gel strength decreased. Among all, 0.3% concentration of Carbopol gel showed good homogeneity, viscosity, and maximum spreadability. As a result, it was chosen for further Cur-AgNP filled gel production.

TABLE 1: COMPOSITION AND EVALUATION OF PLAIN GEL

Code	Amount of Carbopol (%)	Physical aspects	pH	Viscosity (Poise)	Spreadability (gcm/s)	Gel Strength g.s
CG1	0.2	Thin, uniform, translucent, smooth, and clear	8.38 ±0.10	14320±20	14.48±3.30	4.2
CG2	0.3	Thin, uniform, translucent, smooth, and clear	6.58±0.20	19940±87.17	18.25±2.63	4.6
CG3	0.4	Thick, homogenous, transparent, translucent, and smooth	6.07±0.10	29783.33±98.65	10.51±0.92	3.6
CG4	0.5	Thick, homogenous, transparent, translucent, and smooth	7.08±0.05	29790±137.47	7±0.27	3.1

Evaluation of Gel Loaded with Cur-AgNPs: After the preparation and optimization of the plain gel, the synthesized Cur-AgNPs were loaded into the optimized gel formulation (0.3% Carbopol). The prepared gel formulation **Fig. 4B** was further evaluated for different parameters, including appearance, viscosity, pH, and spreadability **Table 2.** A smooth, translucent, clear, and homogenous gel was formed with good homogeneity, viscosity, as well as spreadability.

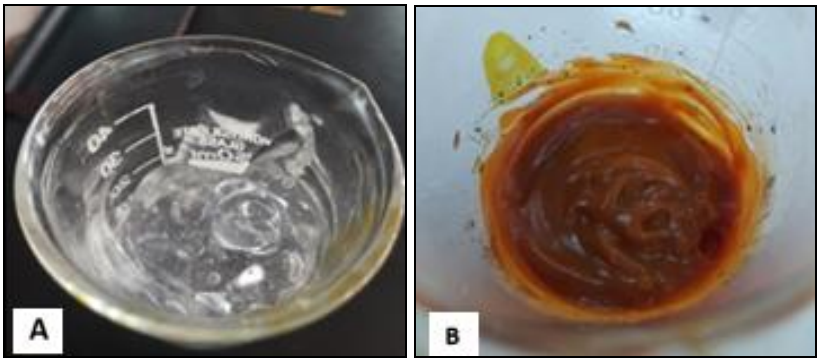

FIG. 4: FORMULATION OF GEL. (A) PLAIN GEL, (B) NPS LOADED GEL

TABLE 2: EVALUATION OF CUR-AgNPs LOADED GEL

Physical aspects	pH	Viscosity (Poise)	Spreadability (gcm/s)
Homogeneous, transparent, smooth, and clear	7.4±0.26	96732±58.32	14.33±3.05

Determination of *In-vitro* Antimicrobial Activity: Using the Kirby-Bauer disc diffusion technique, the antibacterial activity of the Cur-silver nanogel was assessed against the standard test pathogens, *S. aureus*, and the zone of inhibition was calculated. Marketed plain silver nitrate gel was used as a standard to compare the effectiveness of the formulated nanogel. For a dose of 100ug/ml,

the zone of inhibition for Cur-Silver nanogel was found to be more, *i.e*., 2.1 cm, as compared to the marketed silver nitrate gel (1.3 cm). Hence, the silver nanogel made with curcumin had a stronger antibacterial effect than the standard silver nitrate gel **Fig. 5.**

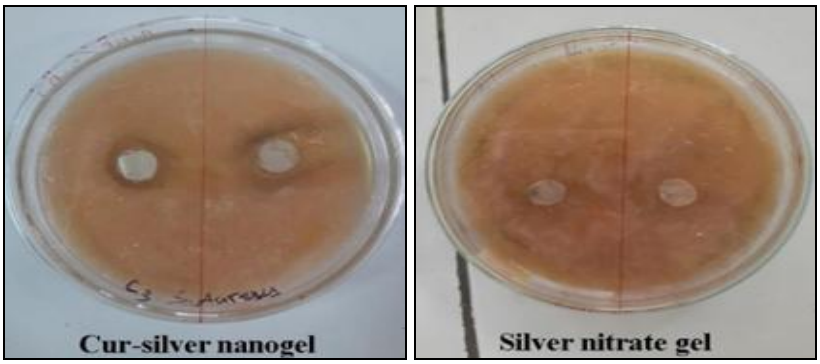

FIG. 5: ZONE OF INHIBITION OF CUR-SILVER NANOGEL AND SILVER NITRATE GEL

***In-vivo* Wound Healing Property:**

Excision Wound Model: The wound contraction area showed no observable difference ($p>0.05$) between the groups receiving plain gel and the negative control group (untreated group). On days 7, 14, and 21, when the animals treated with 0.2% silver nitrate gel and 0.2% gel loaded with Cur-AgNPs was compared to the equivalent untreated control group (negative control and negative control treated with plain gel), the wound area decreased significantly ($p<0.05$, **Fig. 7**). However, it was found that there was a considerable contraction in the wound area from days 7 to 21 when the proportion of wound contraction was considered, using 0.2% silver nitrate gel and 0.2% Cur-AgNPs loaded gel increasing their capacity for healing. In comparison to the silver nitrate gel, the 0.2% Cur-AgNPs-loaded gel exhibited the highest percentage of wound shrinkage (96.96%) as shown in **Table 3**. In **Fig. 6**, the photographs show the wound contraction rate for all the groups of rats in different time periods.

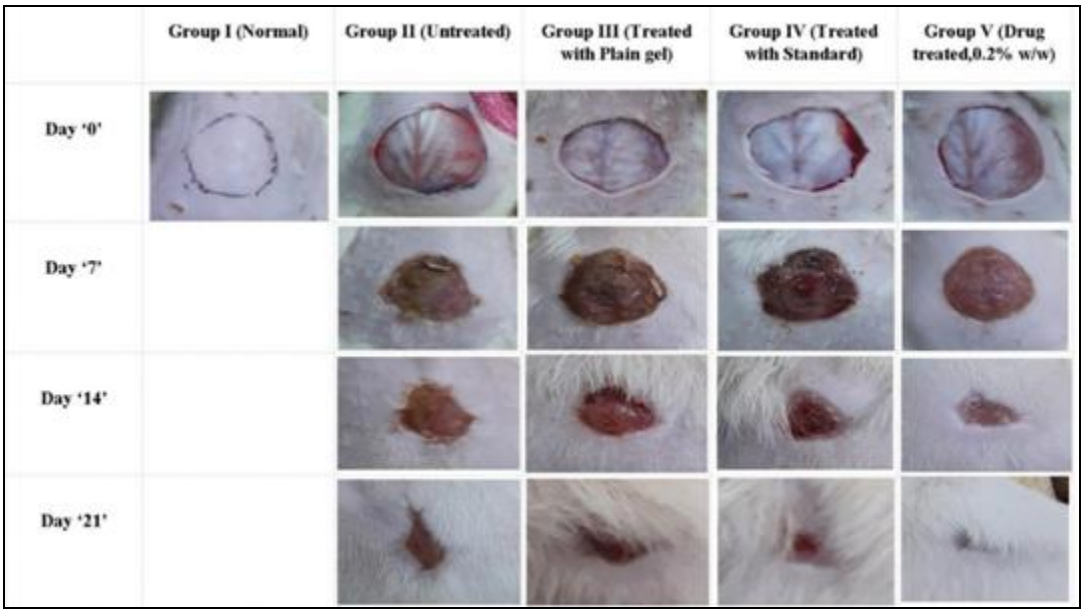

FIG. 6: IMAGES OF WOUND HEALING IN A RAT EXCISION WOUND MODEL AT VARIOUS TIME INTERVALS

TABLE 3: CALCULATION OF THE MEAN, SEM AND % CONTRACTION OF THE WOUND

Experiment group	Day '0'		Day '7'		Day '14'		Day '21'	
	mean SEM	% contraction of wound	mean SEM	% contraction of wound	mean SEM	% contraction of wound	mean SEM	% contraction of wound
Group II (Negative control)	3.92± 0.14	0%	2.72± 0.25	31.7%	1.184± 0.30	70.04%	0.646± 0.36	83.65%

Group III (Treated with plain gel)	4.084± 0.08	0%	2.82± 0.30	30.95%	1.132± 0.32	72.04%	0.648± 0.31	84.13%
Group IV (Standard, 0.2% $AgNO_3$ gel)	4.014± 0.07	0%	2.302± 0.17	42.65%	0.65± 0.13	83.80%	0.184± 0.14	95.41%
Group V (0.2% Cur-AgNP loaded gel)	4.018± 0.22	0%	2.284± 0.24	43.15%	0.64± 0.22	84.07%	0.122± 0.08	96.96%

Histopathological Analysis: From the normal control group's excised tissue, it was visible that the outer epidermis was composed of well-organized epithelial cells (EC). Formation of collagen fibers (CF) and hair follicles (HF) was also found to be normal **Fig. 7A**. The tissue obtained from the untreated group **Fig. 7B** shows that there is severe hyperplasia of the epithelial layer.

The number of stratified squamous epithelial cells increases with magnification. A modest quantity of inflammatory cells is present, and the quantity of sebaceous glands is increasing. The tissue obtained from the plain gel-treated group shows that there is moderate hyperplasia at the epithelial layer with the presence of a few infiltrating cells. There is also a mild increase in the number of stratified squamous cells **Fig. 7C.** In the standard i.e., 0.2% w/w silver nitrate gel **Fig. 7D**, we can see that the lining epithelium is almost similar to the normal intact cells.

The number of hair follicles seems to slightly increase. There is a depletion of collagen fibers in some areas. In the case of 0.2% w/w Cur-AgNP gel, moderate hyperplasia of the epithelium can be seen. There is a depletion of collagen fibers in a few areas. Formation of new hair follicles can also be seen, as shown in **Fig. 7E.**

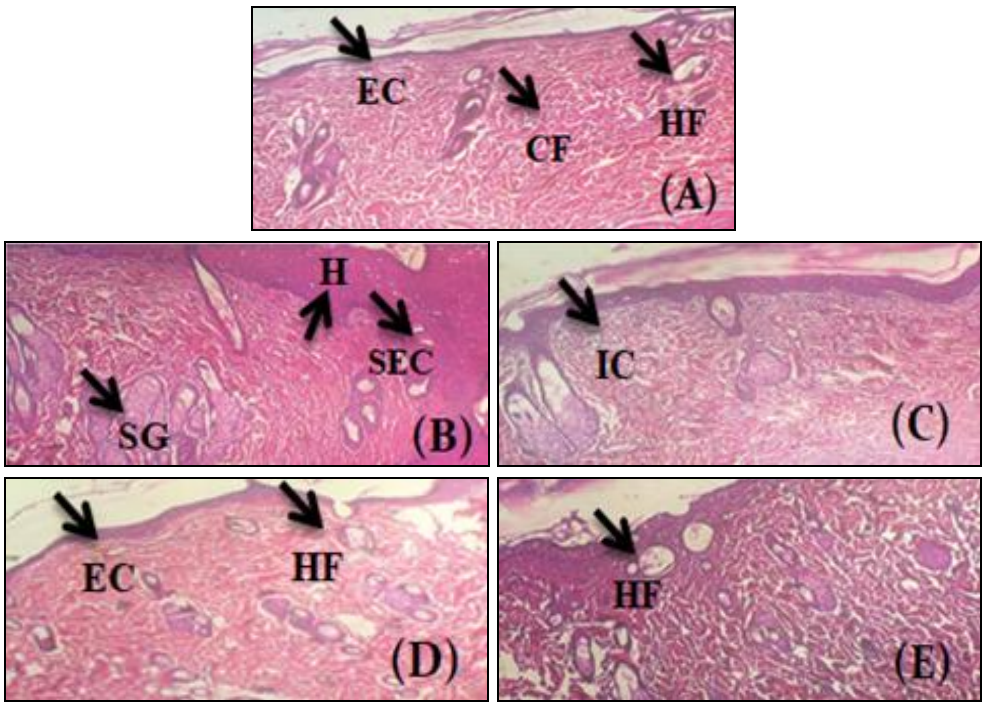

FIG. 7: HISTOPATHOLOGY ANALYSIS OF (A) NORMAL CONTROL GROUP, (B) NEGATIVE CONTROL GROUP (UNTREATED), (C) NEGATIVE CONTROL(PLAIN GEL), (D) MARKETED SILVER NITRATE GEL (0.2%), (E) 0.2%W/W CUR-AgNPs GEL. Arrow represents: EC: Epithelial cells, HF: Hair follicles, CF: Collagen fibers, SG: Sebaceous glands, SEC: Stratified epithelial cells, IC: Infiltrating cells.

Statistical Analysis A two-way statistical analysis of variance (ANOVA) was conducted, and Bonferroni's post-test was used to account for multiple comparisons across groups. **Fig. 8** shows the analysis graph of different groups.

For each group, the results are displayed as Mean S.E.M. with n = 6. *$p<0.05$ indicates statistical significance when compared to the negative control; #$p<0.05$ indicates statistical significance when compared to plain gel.

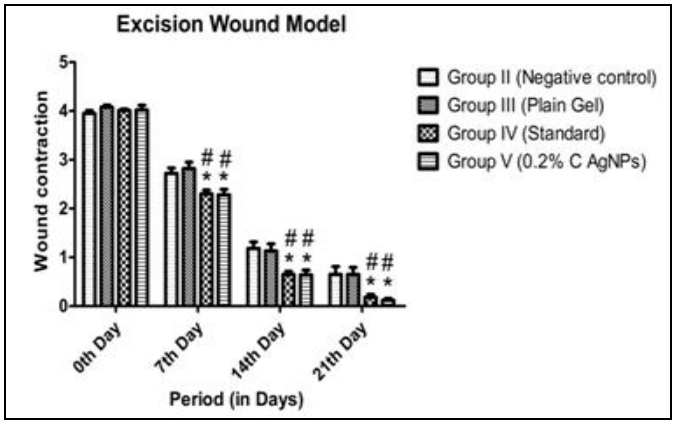

FIG. 8: STATISTICAL ANALYSIS GRAPH OF DIFFERENT GROUPS

CONCLUSION: Curcumin silver nanoparticle was prepared chemically by using a precursor silver nitrate and a reducing agent, sodium citrate, and then it was loaded in carbopol based gel. The nanoparticles were found to be smaller with good stability data, spherical shape, and crystalline nature. pH of nanogel was found as 7.4±0.26, viscosity 96732±58.32cps, with good spreadability at 14.33±3.05 gcm/s. The antimicrobial effect of Cur-AgNPgel was found to be more prominent than the effect of standard silver nitrate gel. The excision wound model's wound healing activities revealed that the Cur-AgNPs loaded gel showed excellent healing activity as compared to the standard silver nitrate gel. Hence, it is clear that Cur-AgNPs showed significant wound healing activity in experimental rats.

It is clear from the investigation that the produced Cur-AgNPs are quite stable. The Cur-AgNPs gel has stable and good physicochemical characteristics. The above results confirmed the effectiveness and enhanced topical delivery of the Cur-AgNPs gel, which could be better than the marketed preparation.

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CONFLICTS OF INTEREST: There are no conflicts of interest in this article.

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