RESEARCH PAPER

DEVELOPMENT, PHYSICOCHEMICAL CHARACTERIZATION AND *IN-VIVO* ANTI-INFLAMMATORY EVALUATION OF *COCCULUS HIRSUTUS* EXTRACT-MEDIATED SILVER NANOPARTICLES IN TNCB-INDUCED CHRONIC DERMATITIS

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ABSTRACT:

Background: Chronic inflammatory skin conditions involve sustained immune activation, oxidative damage, and increased production of inflammatory mediators, resulting in progressive alterations in skin structure and function. Plant-assisted nanotechnology may provide an alternative strategy for developing topical formulations with potential therapeutic benefits.

Objective: This study aimed to synthesize silver nanoparticles using methanolic extract of *Cocculus hirsutus* (MECH), incorporate the optimized nanoparticles into a topical gel, and investigate their anti-inflammatory potential in experimentally induced chronic dermatitis in Wistar albino rats.

Methods: Whole-plant material of *C. hirsutus* was extracted successively using solvents of increasing polarity. Preliminary phytochemical screening was performed, and the methanolic extract was selected for silver nanoparticle synthesis. The prepared nanoparticles were characterized by UV-visible spectroscopy, dynamic light scattering, polydispersity index, zeta potential, and scanning electron microscopy. The optimized nanoparticle dispersion was incorporated into a Carbopol 940-based gel and assessed for physicochemical properties, including pH, viscosity, spreadability, extrudability, homogeneity, and stability. In-vivo evaluation involved eight groups of Wistar albino rats. Dermatitis severity, skin thickness, inflammatory cytokines, oxidative stress markers, and histopathological alterations were considered to assess treatment response.

Conclusion: This investigation establishes a formulation and evaluation approach for MECH-mediated silver nanoparticles incorporated into a topical gel. The combined assessment of formulation characteristics and pharmacological parameters is intended to determine its potential in chronic inflammatory skin conditions. Further studies confirming efficacy, dermal safety, and nanoparticle toxicity are required before clinical translation.

Keywords: *Cocculus hirsutus*; Green synthesis; Silver nanoparticles; Topical gel; Chronic dermatitis; Anti-inflammatory activity; Oxidative stress.

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INTRODUCTION:

Chronic inflammatory skin disorders represent a group of persistent dermatological conditions characterized by recurrent inflammation, disruption of epidermal barrier function, erythema, oedema, dryness, scaling, itching and alterations in normal skin architecture. These conditions develop through complex interactions involving epidermal cells, immune responses, inflammatory mediators and environmental factors. Persistent inflammatory stimulation can promote the release of pro-inflammatory cytokines, including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β), which contribute to inflammatory-cell recruitment, epidermal hyperplasia and tissue damage. Oxidative stress may further aggravate the inflammatory response through excessive generation of reactive oxygen species and depletion of endogenous antioxidant defence mechanisms. Consequently, the regulation of inflammatory mediators and oxidative imbalance

represents an important therapeutic consideration in the management of chronic inflammatory skin conditions [1, 2].

The management of chronic inflammatory skin disorders generally involves topical corticosteroids, calcineurin inhibitors, moisturizers and other anti-inflammatory agents, selected according to disease severity and patient requirements. Topical corticosteroids are commonly used to control inflammatory manifestations, whereas calcineurin inhibitors such as tacrolimus provide an alternative approach through the modulation of T-cell activation and inflammatory signalling. Although these treatments are clinically useful, prolonged or inappropriate use may be associated with local adverse effects, treatment limitations and recurrence of symptoms after discontinuation. In addition, the effectiveness of topical therapy can be influenced by formulation characteristics, drug penetration, skin-barrier condition and patient adherence. These considerations have encouraged research into alternative topical delivery systems and plant-derived bioactive compounds with potential anti-inflammatory and antioxidant properties [3].

Medicinal plants have attracted considerable attention as sources of pharmacologically active constituents, including alkaloids, flavonoids, phenolic compounds, tannins and terpenoids. These phytoconstituents may exhibit antioxidant, antimicrobial and anti-inflammatory activities through different biological mechanisms. *Cocculus hirsutus* (L.) W.Theob., belonging to the family Menispermaceae, is a medicinal climbing plant traditionally used in different regions of South Asia for several health-related conditions, including skin disorders. Phytochemical investigations have reported the presence of alkaloids, flavonoids, triterpenoid derivatives and other secondary metabolites in different parts of the plant. Reported alkaloids include hirsutine, cohirsitine and related isoquinoline derivatives, while flavonoids such as rutin, liquiritin and quercetin have also been described [4]. The diversity of its reported phytoconstituents provides a scientific basis for further pharmacological investigation of this plant.

Previous experimental studies have demonstrated anti-inflammatory potential in extracts of *C. hirsutus*. Sengottuvelu and colleagues investigated the methanolic leaf extract for analgesic and anti-inflammatory activity using in-vitro and animal models, including membrane-stabilization, carrageenan-induced paw oedema and cotton-pellet granuloma methods [5]. Other investigations have also reported anti-inflammatory activity of the plant extracts in experimental models [6]. More recent mechanistic research has explored the ability of *C. hirsutus* extract to influence inflammatory signalling pathways, including Src- and Syk-associated mechanisms [7]. Nevertheless, the available evidence is not sufficient to establish its therapeutic effectiveness in chronic inflammatory skin disorders. In particular, further research is required to investigate the topical application of the methanolic extract, its formulation into suitable delivery systems and its effects on skin-associated inflammatory and oxidative-stress parameters.

Nanotechnology-based drug delivery systems offer opportunities to improve the formulation and localized administration of pharmacologically active compounds. Silver nanoparticles (AgNPs) have been widely investigated because of their distinctive physicochemical properties and reported antimicrobial and biological activities. Their potential applications include topical pharmaceutical preparations, wound-care products and other biomedical formulations [8, 9]. Plant extracts can participate in nanoparticle synthesis by providing phytochemical constituents that contribute to the reduction of silver ions and stabilization of the resulting particles. The phytochemical components associated with the nanoparticle surface may also influence particle characteristics and interactions with biological systems. However, the properties and biological performance of AgNPs depend on several factors, including particle size, size distribution, surface charge, morphology, surface coating and concentration. Therefore, systematic physicochemical characterization and safety evaluation are essential before considering their pharmaceutical application [10,11].

The incorporation of plant-mediated AgNPs into a topical gel provides a practical approach for localized application to affected skin areas. A gel-based formulation can offer convenient application, suitable spreadability and prolonged contact with the application site. Carbopol 940 is commonly used as a pharmaceutical gelling polymer because of its viscosity-building and gel-forming properties, while glycerin can contribute humectant characteristics. Triethanolamine facilitates polymer neutralization and gel formation. Nevertheless, the incorporation of nanoparticles into a semisolid formulation requires careful evaluation of homogeneity, pH, viscosity, spreadability, extrudability and physical stability. Furthermore, the interaction between the nanoparticle dispersion and the gel matrix may influence particle aggregation, distribution and overall formulation performance. Accordingly, the development of a suitable topical gel should be supported by appropriate physicochemical characterization and stability assessment [12,13].

Despite the reported pharmacological activities of *C. hirsutus* and the increasing interest in plant-mediated silver nanoparticles, the development and evaluation of *C. hirsutus* extract-mediated silver nanoparticles in a topical gel for experimental chronic dermatitis remain insufficiently

investigated. The combination of plant-derived phytochemicals and a nanoparticle-based delivery system may provide an approach for exploring localized anti-inflammatory activity; however, the individual contribution of the plant extract, nanoparticle component and gel vehicle requires systematic evaluation. In addition, the assessment of clinical dermatitis manifestations together with inflammatory cytokines and oxidative-stress biomarkers can provide a broader understanding of the pharmacological response. [13,14,15].

Therefore, the present investigation was designed to develop and physicochemically characterize silver nanoparticles mediated by the methanolic extract of *Cocculus hirsutus* (MECH-AgNPs), incorporate the optimized nanoparticle dispersion into a Carbopol-based topical gel and evaluate its anti-inflammatory potential in an experimentally induced chronic dermatitis model in Wistar albino rats [16,17]. The study included preliminary phytochemical screening, nanoparticle characterization, formulation optimization, evaluation of gel properties and stability, and in-vivo assessment using clinical dermatitis scoring, skin-thickness measurement, inflammatory cytokine estimation (TNF-α, IL-6 and IL-1β) and oxidative-stress parameters (MDA, GSH, SOD and CAT). Tacrolimus 0.1% ointment was included as the standard reference treatment. The investigation was intended to explore the potential of MECH-mediated silver nanoparticles as a topical formulation and generate preliminary experimental evidence regarding their anti-inflammatory performance.

MATERIALS AND METHODS:

MATERIALS:

The whole plant of *Cocculus hirsutus* was collected from the Satpura region of Maharashtra, India, and authenticated by the Department of Botany, Bhusawal Arts, Science and P. O. Nahata Commerce College, Bhusawal, Maharashtra. Silver nitrate ($AgNO_3$), Carbopol 940, glycerin, and triethanolamine were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Petroleum ether, chloroform, methanol, and other analytical-grade solvents were obtained from Merck Life Science Pvt. Ltd., Mumbai, India. Tetrachloronitrobenzene (TCNB) was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) and used as the chemical inducer for experimental chronic dermatitis. Tacrolimus 0.1% w/w ointment was procured from Astellas Pharma Inc., Tokyo, Japan, and used as the standard reference treatment. All chemicals and reagents were of analytical grade and used as received.

Collection and Authentication of Plant Material

The whole plant of *Cocculus hirsutus* (L.) W. Theob. (Family: Menispermaceae) was collected from the Satpura region of Maharashtra, India. The plant material was washed thoroughly, shade-dried, pulverized and authenticated by Prof. Dr. S. V. Patil, Department of Botany, Bhusawal Arts, Science and P. O. Nahata Commerce College, Bhusawal, Maharashtra. The powdered material was stored in an airtight container under dry conditions until further use.

Preparation of Plant Extracts

Successive Soxhlet extraction of powdered *C. hirsutus* (100 g) was performed using petroleum ether (60–80°C), chloroform, methanol and distilled water in increasing order of polarity, maintaining a drug-to-solvent ratio of 1:10 (w/v). Each extraction was continued for approximately 6–8 hours. The extracts were filtered and concentrated separately using a rotary vacuum evaporator, while the aqueous extract was concentrated using a suitable low-temperature technique. The dried extracts were weighed to determine percentage yield and stored in amber-coloured containers at 2–8°C until further investigation [18, 19, 20].



Fig. 1: Extraction of *Cocculus hirsutus* (L.) by Soxhlet Apparatus

Preliminary Phytochemical Screening

The petroleum ether, chloroform, methanol and aqueous extracts were subjected to preliminary qualitative phytochemical screening using standard procedures. The extracts were examined for alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, cardiac glycosides and carbohydrates using appropriate chemical tests, including Dragendorff's, Mayer's, Wagner's, Shinoda, ferric chloride, foam, Salkowski, Liebermann–Burchard, Keller–Killiani and Molisch's tests, respectively [20, 21].

FORMULATION DEVELOPMENT:

Selection of Excipients

The excipients used for the development of *Cocculus hirsutus* methanolic extract-mediated silver nanoparticle gel (MECH-AgNP gel) were selected based on their pharmaceutical acceptability, compatibility with the nanoparticle dispersion and suitability for topical application. In the preceding phytochemical investigation, the methanolic extract of *C. hirsutus* (MECH) was selected for further studies because it exhibited a comparatively rich phytochemical profile,

particularly with respect to alkaloids, flavonoids, phenolic compounds and tannins. Therefore, MECH was used as the plant-derived component for silver nanoparticle synthesis. Carbopol 940 was selected as the primary gelling agent because of its ability to produce a homogeneous hydrogel with suitable viscosity and consistency. Glycerin was used as a humectant to improve moisture retention and the application properties of the formulation. Triethanolamine (TEA) was selected as a neutralizing agent to facilitate gel formation by adjusting the pH of the hydrated Carbopol 940 dispersion. Purified water was used as the aqueous vehicle. The optimized MECH-mediated silver nanoparticle dispersion was incorporated as the active nanoparticle component of the final topical formulation [22, 23].

Solubility and Compatibility Studies of Excipients

The solubility and dispersibility of the selected excipients were evaluated before the preparation of the final MECH-AgNP gel formulation. Accurately weighed quantities of Carbopol 940 were gradually dispersed in purified water under continuous stirring to evaluate their hydration behaviour, uniformity and tendency to form lumps. Glycerin was examined for its miscibility with the aqueous vehicle, whereas triethanolamine was diluted separately with purified water before its incorporation into the polymeric dispersion. The prepared mixtures were visually examined for uniformity, undissolved particles, precipitation and phase separation [24,25]. Compatibility studies were subsequently performed between the optimized MECH-mediated silver nanoparticle dispersion and the selected gel-forming excipients. The nanoparticle dispersion was separately mixed with Carbopol 940 and glycerin, and the resulting mixtures were examined for changes in colour, aggregation, precipitation and physical appearance. Fourier-transform infrared spectroscopy (FTIR) was used, where applicable, to investigate possible interactions between the phytochemical constituents associated with the MECH-mediated silver nanoparticles and the selected formulation components. The influence of the gel base on particle size, polydispersity index (PDI) and zeta potential was also assessed wherever technically feasible. The excipient system demonstrating satisfactory compatibility and acceptable physical characteristics was selected for further development of the topical gel [26,27]

Formulation Development of *Cocculus hirsutus* Methanolic Extract-Mediated Silver Nanoparticle Gel

The topical silver nanoparticle gel was prepared using the optimized silver nanoparticle dispersion synthesized with the methanolic extract of *C. hirsutus* (MECH). Initially, the required quantity of Carbopol 940 was accurately weighed and gradually sprinkled over a portion of purified water under continuous mechanical stirring to obtain uniform dispersion of the polymer. The dispersion was allowed to hydrate adequately until a uniform polymeric system was obtained. Subsequently, the required quantity of glycerin was added to the hydrated Carbopol 940 dispersion and mixed thoroughly to obtain a uniform gel base [28,29,30]

The optimized MECH-mediated silver nanoparticle dispersion was then gradually incorporated into the hydrated polymeric base under continuous gentle stirring to ensure uniform distribution of the nanoparticles throughout the gel matrix. The mixture was stirred carefully to obtain a homogeneous dispersion while minimizing air entrapment and nanoparticle aggregation. Triethanolamine was diluted with purified water and added dropwise to the formulation under continuous stirring until the desired gel consistency and suitable topical pH were achieved. The final weight of the formulation was adjusted using purified water, and the mixture was stirred until a uniform semisolid gel was obtained. The prepared MECH-AgNP gel was allowed to stand to facilitate the removal of entrapped air bubbles. Finally, the formulation was transferred into clean, well-closed, light-protective containers and stored under controlled conditions until further evaluation [31,32]

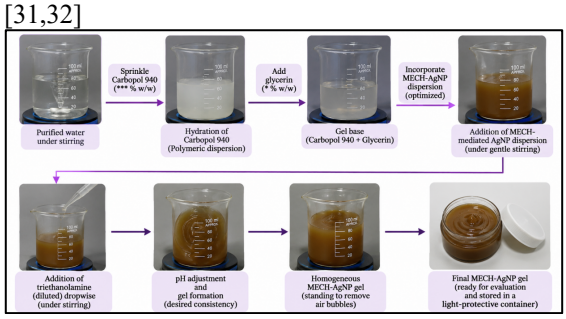

Fig. 2: Schematic representation of the formulation development and preparation of Cocculus hirsutus methanolic extract-mediated silver nanoparticle gel (MECH-AgNP gel)

Optimization and Selection of the Final Gel Formulation

Different batches of MECH-mediated silver nanoparticle gel were prepared by varying the concentration of Carbopol 940 and, where applicable, the concentration of the incorporated MECH-AgNP dispersion. The influence of polymer concentration on gel consistency, viscosity, spreadability, extrudability and homogeneity was investigated. The prepared formulations were evaluated for their physical appearance, colour, pH, homogeneity and uniformity of nanoparticle distribution. The optimized formulation was selected based on its acceptable physicochemical characteristics, suitable topical pH, appropriate viscosity, ease of application, spreadability and physical stability. The selected MECH-AgNP gel formulation was subsequently subjected to stability

studies under predefined storage conditions. Changes in physical appearance, colour, pH, viscosity, homogeneity and nanoparticle aggregation were recorded at predetermined intervals to assess the stability of the developed topical formulation [33]

Physicochemical Characterization of MECH-Mediated Silver Nanoparticles [34,35,36]

Visual Observation and Colour Change

The formation of silver nanoparticles using the methanolic extract of *Cocculus hirsutus* (MECH) was initially monitored by visual observation of the reaction mixture. The colour of the reaction mixture was recorded before and after the addition of the silver nitrate solution. The development of a characteristic yellowish-brown to dark-brown colour was considered a preliminary indication of silver nanoparticle formation. The observations were further confirmed using UV–Visible spectroscopy.

Particle Size and Polydispersity Index (PDI)

The average hydrodynamic particle size and polydispersity index of the synthesized MECH-mediated silver nanoparticles were determined using a dynamic light scattering (DLS)-based particle size analyser. The nanoparticle dispersion was suitably diluted with purified water to obtain an appropriate concentration for analysis. The diluted sample was transferred into a clean measurement cuvette and analysed using the instrument. The average hydrodynamic diameter and PDI were recorded to evaluate particle-size distribution and dispersion uniformity.

Zeta Potential Analysis

The surface charge of the synthesized silver nanoparticles was evaluated by determining the zeta potential using a suitable zeta potential analyser. The nanoparticle dispersion was diluted with an appropriate dispersing medium and transferred into a suitable measurement cell. The zeta potential was measured using electrophoretic light scattering, and the resulting value was recorded to assess the surface-charge characteristics and potential colloidal stability of the nanoparticle dispersion.

Morphological Characterization

The surface morphology and structural characteristics of the synthesized silver nanoparticles were examined using scanning electron microscopy (SEM). For SEM analysis, the purified nanoparticle sample was appropriately dried, mounted on a suitable sample holder and coated with a conductive material when required. The obtained micrographs were analysed to determine the particle shape, surface morphology and approximate particle dimensions.

Evaluation of the Final MECH-AgNP Gel Formulation [37,38]

Physical Appearance and Organoleptic Evaluation

The prepared MECH-AgNP gel formulation was evaluated for its physical appearance and organoleptic characteristics, including colour, odour, consistency and general appearance. The formulation was visually examined for uniformity, visible particulate matter, phase separation, aggregation and any other physical changes. The observations were recorded to assess the overall acceptability of the prepared topical gel.

Homogeneity

The homogeneity of the prepared gel was evaluated by visual inspection and by applying a small quantity of the formulation between two clean glass slides. The formulation was examined for the presence of lumps, coarse particles, aggregates and visible inconsistencies. The uniformity of the gel was assessed to determine whether the incorporated MECH-mediated silver nanoparticles were evenly distributed throughout the formulation.

Determination of pH

The pH of the prepared MECH-AgNP gel was determined using a calibrated digital pH meter. Approximately 2.5 g of gel was accurately weighed and dispersed in 25 mL of purified water with gentle stirring to obtain a uniform dispersion. The pH electrode was immersed in the prepared sample, and the pH value was recorded after stabilization of the instrument reading. The measurement was performed in triplicate, and the results were expressed as mean ± standard deviation.

Viscosity and Rheological Evaluation

The viscosity of the prepared gel was determined using a Brookfield rotational viscometer equipped with an appropriate spindle. A suitable quantity of the formulation was transferred into the sample container, and the viscosity was measured at a predetermined temperature and rotational speed. The instrument reading was recorded after stabilization. The viscosity was expressed in centipoise (cP) or mPa·s. Where applicable, rheological behaviour was investigated at different rotational speeds to evaluate the flow characteristics of the gel formulation.

Spreadability

The spreadability of the prepared gel was determined using the glass-slide method. Approximately 1 g of the gel was placed between two clean glass slides. A predetermined weight was placed over the upper slide for a fixed period to obtain a uniform gel layer. Subsequently, a specified weight was attached to the upper slide, and the time required for the slide to move through a predetermined distance was recorded. Spreadability was calculated using:

$$S = (M \times L) / T \quad \text{…….. (Eq. 1)}$$

Where, S represents spreadability, M represents the applied weight, L represents the distance travelled by the slide and T represents the time required.

Extrudability

The extrudability of the prepared gel was evaluated by filling a suitable collapsible tube with a known quantity of the formulation. The tube was closed properly, and a predetermined force was applied to extrude the gel through its nozzle. The quantity of gel extruded under the specified conditions was recorded. The ease of extrusion and consistency of the extruded gel were examined to assess the suitability of the formulation for topical application.

Gel Content Uniformity

The uniformity of the incorporated silver nanoparticle component was evaluated by collecting samples from different portions of the prepared gel formulation. The samples were appropriately processed to determine the silver content using a validated analytical method, such as atomic absorption spectroscopy (AAS) or inductively coupled plasma optical emission spectroscopy (ICP-OES), where available. The results were compared to assess the uniform distribution of silver within the gel matrix. The silver content was expressed relative to the theoretical or specified formulation content.

Stability Studies

The optimized MECH-AgNP gel formulation was subjected to stability studies under predefined storage conditions. The samples were stored in well-closed, light-protective containers and examined at predetermined intervals. The physical appearance, colour, odour, pH, viscosity, homogeneity and evidence of aggregation or phase separation were recorded throughout the study. Where appropriate, particle size and zeta potential of the recovered nanoparticle dispersion were also assessed to investigate changes in nanoparticle characteristics during storage. The results were compared with the initial observations to evaluate the physical and physicochemical stability of the developed topical gel.

***In-vivo* Pharmacological Evaluation**

The *in-vivo* study was conducted to evaluate the safety and anti-inflammatory potential of *Cocculus hirsutus* methanolic extract (MECH) and its silver nanoparticle gel (MECH-AgNP gel) against experimentally induced chronic dermatitis in Wistar albino rats. The evaluation included toxicity assessment, clinical examination, and biochemical analysis [39, 40, 41]

Procurement and Maintenance of Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing 150–200 g, were procured from a CPCSEA-registered animal breeding facility (National Institute of Biosciences, Pune, Maharashtra). The animals were maintained in the institutional animal house of TVES's Hon. Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur, Jalgaon, Maharashtra, under standard laboratory conditions (22 ± 3°C, 50–60% relative humidity and a 12-hour light/dark cycle). Standard pellet diet and filtered drinking water were provided ad libitum. The animals were acclimatized for seven days before the experiments. All experimental procedures were conducted in accordance with CPCSEA guidelines after prior approval from the Institutional Animal Ethics Committee (IAEC Approval No.: IAEC/04/25/Proposal No. 02) [42].

Preliminary Dermal Tolerability Evaluation

The preliminary dermal tolerability of the *Cocculus hirsutus* methanolic extract-mediated silver nanoparticle gel (MECH-AgNP gel) was evaluated before pharmacological assessment. The formulation was assessed for local skin irritation and general tolerability in accordance with the IAEC-approved protocol. The treated skin was examined for erythema, oedema, dryness, scaling, excoriation and ulceration. General behaviour, food and water intake, and body weight were monitored throughout the observation period.

The MECH-AgNP gel formulations containing 0.5%, 1.0% and 2.0% w/w extract-equivalent concentrations were evaluated. The quantity applied per unit skin area, application frequency and observation period were recorded. The skin reactions were documented using a consistent observation system, and the findings were used to assess the local tolerability of the formulations [43,44]

Induction of Experimental Chronic Dermatitis Using Tetrachloronitrobenzene (TCNB)

Experimental chronic dermatitis was induced in Wistar albino rats using tetrachloronitrobenzene (TCNB), following the institutionally approved experimental protocol. The exact chemical identity and positional isomer of TCNB were verified using the manufacturer's product label and certificate of analysis before preparation of the test solution. The TCNB preparation was administered topically to the designated skin area according to the approved sensitization and repeated-exposure procedure. The concentration, vehicle, application volume, treated skin area, application frequency and total induction period were maintained as specified in the IAEC-approved protocol. The development of dermatitis was monitored through regular examination of the skin for erythema, oedema, dryness, scaling, excoriation and skin thickening. After completion of the induction period, the animals were examined to confirm the development of visible dermatitis before initiation of the treatment phase. The same anatomical region was used for disease induction and subsequent assessment wherever specified in the approved protocol.[42,43]

Experimental Design and Animal Grouping

Forty-eight Wistar albino rats were divided randomly into eight experimental groups, with six animals in each group (n = 6). The experimental groups were organized as follows:

Table 1: Experimental design and grouping for evaluation of anti-inflammatory activity of MECH

Group	Treatment
Group I	Normal control: No TCNB induction; vehicle treatment
Group II	Disease control: TCNB induction; vehicle treatment
Group III	Blank gel control: TCNB induction; Carbopol gel base without MECH or AgNPs
Group IV	Standard control: TCNB induction; Tacrolimus 0.1% w/w ointment
Group V	MECH extract gel: TCNB induction; MECH gel 1.0% w/w
Group VI	MECH-AgNP gel low concentration: 0.5% w/w extract-equivalent concentration
Group VII	MECH-AgNP gel medium concentration: 1.0% w/w extract-equivalent concentration
Group VIII	MECH-AgNP gel high concentration: 2.0% w/w extract-equivalent concentration

The normal-control group was maintained without TCNB induction. All other groups underwent dermatitis induction according to the approved protocol. The treatment groups received the respective topical formulations following confirmation of dermatitis. The MECH-AgNP concentrations were expressed as extract-equivalent concentrations in the final gel. The actual silver content of the nanoparticle formulations was determined separately and reported in terms of silver concentration.

Topical Treatment Schedule

After successful induction of experimental dermatitis, topical treatment was administered to the respective experimental groups. Group IV received tacrolimus 0.1% w/w ointment as the standard reference treatment. Group V received MECH extract gel at 1.0% w/w, while Groups VI, VII and VIII received MECH-AgNP gel containing 0.5%, 1.0% and 2.0% w/w extract-equivalent concentrations, respectively. Group III received the blank Carbopol gel base without MECH or silver nanoparticles. Group II received the designated vehicle without active treatment, while Group I received the vehicle without prior TCNB induction. The quantity of each topical formulation was standardized according to the treated skin area and recorded in mg/cm². The application frequency and treatment duration were maintained consistently across the respective treatment groups according to the approved IAEC schedule. The treatment response was assessed at predefined observation intervals by recording clinical skin changes and other relevant pharmacological parameters [47,48]

Evaluation of Anti-Inflammatory and Dermatitis-Improving Activity

The pharmacological activity of MECH-AgNP gel was evaluated using clinical, physical and biochemical parameters [45-49]

Clinical Assessment of Dermatitis

The severity of dermatitis was evaluated by examining erythema, oedema, dryness, scaling and excoriation. Each parameter was scored using a four-point scale:

Table 2: Clinical Assessment Scoring of Dermatitis

Score	Severity	Description
0	Absent	No visible skin alteration
1	Mild	Slight or localized skin alteration
2	Moderate	Clearly visible skin alteration
3	Severe	Marked or extensive skin alteration

Measurement of Skin Thickness

Skin thickness was measured using a calibrated digital thickness-measuring instrument at the same anatomical site throughout the experimental period. Measurements were recorded before and after dermatitis induction and during the treatment period. The changes in skin thickness were compared among the normal-control, disease-control, standard-treatment and test-formulation groups.

Monitoring of Body Weight and General Behaviour

Body weight was recorded at baseline and at predefined intervals throughout the experimental period. General behaviour, food and water intake, grooming, activity and overall physical condition were monitored. Any abnormal clinical signs or adverse reactions were documented.

Biochemical Assessment of Inflammatory Markers

At the end of the experimental period, skin tissue samples were collected from the treated areas for biochemical evaluation. The tissues were homogenized in an appropriate ice-cold buffer and centrifuged to obtain the supernatant. The inflammatory mediators tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) were estimated using validated

ELISA kits according to the manufacturers' instructions. The concentrations of the inflammatory markers were calculated from the respective standard curves and expressed in the appropriate units. The results were normalized to tissue weight or total protein content, depending on the validated assay procedure [46,47].

Assessment of Oxidative Stress Markers

The antioxidant and oxidative stress status of the skin tissue was evaluated by estimating malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). MDA levels were determined using the thiobarbituric acid-reactive substances (TBARS) method. Reduced glutathione was estimated using Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). SOD activity was determined using a validated spectrophotometric assay based on inhibition of a superoxide-dependent reaction. Catalase activity was estimated by monitoring the decomposition of hydrogen peroxide spectrophotometrically. The results were expressed in the appropriate units and normalized to total protein content wherever applicable. Changes in oxidative stress markers were compared among all experimental groups [48,49]

Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using appropriate statistical software. Differences among the experimental groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test when the assumptions of the analysis were satisfied.

For parameters measured repeatedly over time, such as skin thickness, an appropriate repeated-measures ANOVA or mixed-effects model was used. Ordinal dermatitis scores were analysed using suitable non-parametric statistical methods. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening

The preliminary phytochemical screening revealed variations in the chemical constituents among the different solvent extracts of *Cocculus hirsutus*. The methanolic extract showed a comparatively rich phytochemical profile, with strong presence of alkaloids, flavonoids, phenolic compounds and tannins, along with moderate levels of saponins, terpenoids, steroids, glycosides and carbohydrates. The aqueous extract showed strong presence of saponins and carbohydrates, whereas the chloroform extract was particularly rich in terpenoids. Petroleum ether showed moderate presence of terpenoids and steroids. Overall, the methanolic extract demonstrated the broadest distribution of the screened phytoconstituents, supporting its selection for further investigation and silver nanoparticle development.

Table 3: Preliminary Phytochemical Screening of Different Extracts of *Cocculus hirsutus*

Phytochemical constituents	Petroleum ether	Chloroform	Methanol	Aqueous
Alkaloids	−	+	+++	++
Flavonoids	−	+	+++	++
Phenolic compounds	−	+	+++	++
Tannins	−	−	+++	++
Saponins	−	−	++	+++
Terpenoids	++	+++	++	+
Steroids	++	++	+	−
Glycosides	−	+	++	++
Carbohydrates	−	−	++	+++

Interpretation: − = absent; + = weak presence; ++ = moderate presence; +++ = strong presence.

FORMULATION DEVELOPMENT:

Solubility and Compatibility Studies of Excipients

The solubility and dispersibility studies were performed to assess the suitability of the selected excipients for developing the MECH-mediated silver nanoparticle gel. Carbopol 940 exhibited good hydration and swelling behaviour in purified water, forming a polymeric dispersion suitable for gel preparation. Glycerin showed complete miscibility with the aqueous vehicle, while triethanolamine was readily miscible with purified water. The MECH-mediated silver nanoparticle dispersion showed uniform incorporation into the aqueous formulation system under controlled mixing conditions.

Table 4: Solubility and Dispersibility Profile of Selected Excipients

Sr. No.	Ingredient	Test concentration (% w/w)	Solubility/dispersibility in purified water	Observation

1	Carbopol 940	0.5–1.5	Dispersible; undergoes hydration	Uniform polymeric dispersion after hydration
2	Glycerin	5.0	Completely miscible	Clear and homogeneous mixture
3	Triethanolamine	0.1–0.5	Completely miscible	Clear aqueous solution
4	MECH-AgNP dispersion	5.0–10.0	Dispersible	Uniform dispersion under gentle stirring
5	Purified water	q.s. to 100	Miscible vehicle	Aqueous formulation medium

The excipient screening indicated that the selected ingredients were suitable for preparing an aqueous topical gel. Carbopol 940 was considered an appropriate gelling polymer because of its hydration and viscosity-building properties. Glycerin contributed humectant properties and improved the expected application characteristics of the formulation. Triethanolamine facilitated polymer neutralization and gel development. The aqueous MECH-AgNP dispersion was incorporated as the active component. These findings supported the selection of Carbopol 940, glycerin, triethanolamine and purified water for further formulation development.

Formulation Development of MECH-AgNP Gel

Nine formulations (F1–F9) were designed by varying the concentrations of Carbopol 940 and MECH-AgNP dispersion while maintaining glycerin at a constant concentration. Triethanolamine was added quantity sufficient to obtain the required gel consistency and suitable pH. Purified water was used to adjust the final formulation weight to 100 g.

Table 5: Composition of MECH-AgNP Gel Formulations (F1–F9)

Ingredients (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 940	0.5	0.5	0.5	0.75	0.75	0.75	1.0	1.0	1.0
Glycerin	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
MECH-AgNP dispersion	5.0	7.5	10.0	5.0	7.5	10.0	5.0	7.5	10.0
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Purified water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Final weight (g)	100	100	100	100	100	100	100	100	100

Note: q.s. = quantity sufficient.

The nine formulations were developed by varying the polymer concentration and the amount of MECH-AgNP dispersion. Carbopol 940 concentrations of 0.5%, 0.75% and 1.0% w/w were selected to investigate their influence on gel consistency, viscosity and spreadability. The MECH-AgNP dispersion was incorporated at 5%, 7.5% and 10% w/w to examine its influence on the physical characteristics and uniformity of the formulations.

The formulation design provided different combinations of polymer concentration and nanoparticle dispersion content. Increasing the concentration of Carbopol 940 was expected to increase the viscosity and consistency of the gel,

whereas increasing the nanoparticle dispersion could influence its appearance, homogeneity and physical stability. The nine formulations were therefore suitable for comparative physicochemical evaluation to identify the formulation with the most appropriate characteristics for topical application.

Physicochemical Characterization of MECH-Mediated Silver Nanoparticles

Visual Observation and Colour Change

The formation of silver nanoparticles using the methanolic extract of Cocculus hirsutus (MECH) was initially monitored through visual observation. The methanolic extract exhibited a yellowish appearance before the addition of silver nitrate solution. Following the addition of silver nitrate, the reaction mixture gradually changed from yellowish to yellowish-brown and finally developed a dark-brown colour. This colour transformation indicated the possible reduction of silver ions (Ag^{+}) into metallic silver nanoparticles (Ag^{0}).

The observed colour change may be attributed to the surface plasmon resonance phenomenon associated with silver nanoparticles. Phytochemical constituents, particularly phenolic and flavonoid compounds present in MECH, may contribute to the reduction and stabilization of the nanoparticles. The preliminary observations were subsequently supported by UV–Visible spectroscopic analysis.

Table 6: Visual observation during the synthesis of MECH-mediated silver nanoparticles

Observation stage	Colour observed
Methanolic extract before reaction	Yellowish
Silver nitrate solution	Colourless
Immediately after mixing	Pale yellow
During reaction	Yellowish-brown
Final reaction mixture	Dark brown

Particle Size and Polydispersity Index

The average hydrodynamic particle size and polydispersity index of the synthesized silver nanoparticles were evaluated using dynamic light scattering. The results indicated particle sizes ranging from 85.6 to 142.8 nm, with PDI values ranging from 0.214 to 0.386. Among the evaluated batches, MECH-AgNP-5 demonstrated the lowest particle size of 85.6 nm and a PDI of 0.214. The comparatively lower PDI indicated a narrower particle-size distribution than the other batches. The results suggested that MECH-AgNP-5 exhibited comparatively favourable dispersion characteristics.

Table 7: Particle size and PDI of MECH-mediated silver nanoparticles

Batch code	Particle size (nm)	PDI	Zeta potential (mV)
MECH-AgNP-1	118.6	0.312	−22.5
MECH-AgNP-2	105.4	0.286	−25.8
MECH-AgNP-3	96.8	0.258	−28.6
MECH-AgNP-4	112.3	0.301	−21.4
MECH-AgNP-5	85.6	0.214	−32.4
MECH-AgNP-6	98.7	0.267	−27.2
MECH-AgNP-7	142.8	0.386	−18.6
MECH-AgNP-8	109.5	0.294	−24.9
MECH-AgNP-9	93.2	0.243	−30.1

Zeta potential analysis

Zeta potential analysis was performed to evaluate the surface-charge characteristics and potential colloidal stability of the synthesized silver nanoparticles. The results demonstrated zeta potential values ranging from −18.6 to −32.4 mV. Among the evaluated batches, MECH-AgNP-5 exhibited a zeta potential of −32.4 mV, indicating a comparatively higher magnitude of surface charge. This may promote electrostatic repulsion between dispersed particles and reduce their tendency to aggregate. The negative surface charge may be associated with phytochemical constituents adsorbed onto the nanoparticle surface.

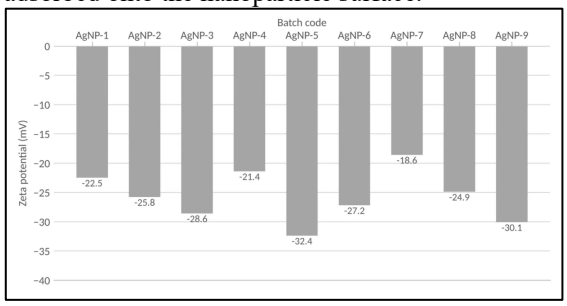

Fig. 3: Zeta Potential Analysis for all batches

Morphological characterization by SEM

The surface morphology of the synthesized MECH-mediated silver nanoparticles was examined using

scanning electron microscopy. The SEM observations showed predominantly spherical to nearly spherical particles with relatively smooth surface characteristics. The particles appeared distributed with occasional aggregation.

The approximate particle dimensions observed through SEM were in the range of 40–90 nm. The smaller dimensions observed by SEM compared with DLS measurements may be attributed to differences in measurement principles. SEM evaluates the dimensions of dried particles, whereas DLS measures the hydrodynamic diameter of particles along with their associated surface-bound molecules and solvation layer.

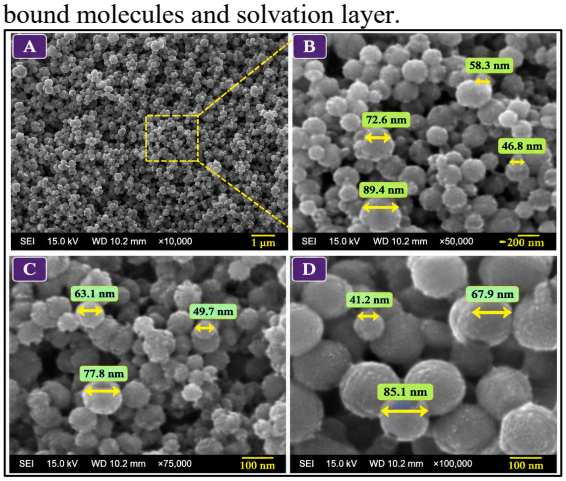

Fig. 4: Scanning electron microscopy (SEM) analysis of MECH-mediated silver nanoparticles

Selection of optimized MECH-mediated silver nanoparticle batch

The optimized batch was selected by considering the combined physicochemical characteristics, including particle size, PDI, zeta potential and morphological appearance. Among the nine batches, MECH-AgNP-5 exhibited a comparatively smaller particle size (85.6 nm), lower PDI (0.214) and higher absolute zeta potential (−32.4 mV). These characteristics indicated a comparatively narrow particle-size distribution and favourable electrostatic stabilization. Therefore, MECH-AgNP-5 was selected as the optimized batch for subsequent incorporation into the topical gel formulation.

Formulation development

Three gel formulations were prepared using different concentrations of Carbopol 940 as the gelling agent. Glycerin was maintained at 5% w/w, while the MECH-AgNP dispersion was incorporated at 7.5% w/w. Triethanolamine was added quantity sufficient to achieve the desired gel consistency and pH.

Table 18: Composition of different MECH-AgNP gel formulations

Ingredients (% w/w)	MG-1	MG-2	MG-3
Carbopol 940	0.50	0.75	1.00
Glycerin	5.00	5.00	5.00
MECH-AgNP dispersion	7.50	7.50	7.50
Triethanolamine	q.s.	q.s.	q.s.
Purified water	q.s. to 100	q.s. to 100	q.s. to 100

Evaluation of the Final MECH-AgNP Gel Formulation

Physical Appearance and Organoleptic Evaluation

All three formulations exhibited a brown colour characteristic of the incorporated MECH-mediated silver nanoparticle dispersion. MG-1 showed a light-brown appearance with a smooth consistency, whereas MG-2 exhibited a uniform brown colour and smooth gel texture. MG-3 demonstrated a comparatively darker appearance and thicker consistency. No visible phase separation or coarse particulate matter was observed in the formulations. MG-2 showed the most desirable overall physical appearance.

Homogeneity

The homogeneity assessment indicated that all three formulations showed acceptable uniformity without visible lumps or coarse aggregates. MG-2 demonstrated excellent homogeneity and uniform distribution of the nanoparticle dispersion throughout the gel matrix. The smooth consistency suggested suitable incorporation of MECH-AgNPs into the Carbopol gel base.

Determination of pH

The pH values of MG-1, MG-2 and MG-3 were 5.82 ± 0.04, 6.18 ± 0.03 and 6.42 ± 0.05, respectively. The results indicated a gradual increase in pH with increasing Carbopol concentration under the formulation conditions. MG-2 exhibited a pH of 6.18, indicating a suitable mildly acidic to near-neutral formulation characteristic for further topical evaluation. Actual skin compatibility should be established through appropriate tolerability testing.

Viscosity and Rheological Evaluation

The viscosity values of MG-1, MG-2 and MG-3 were 18,500 ± 320, 26,400 ± 410 and 34,800 ± 520 cP, respectively. An increase in viscosity was observed with increasing Carbopol 940 concentration. MG-3 exhibited the highest viscosity, which may affect its ease of application and extrusion. MG-2 demonstrated an intermediate viscosity, providing a suitable balance between gel consistency and handling characteristics.

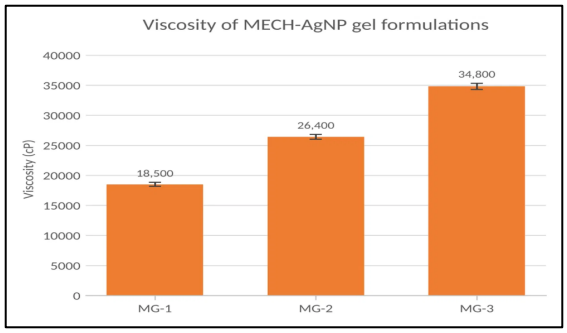

Fig. 5: Viscosity of MECH-AGNP gel fprmulations

Spreadability

The spreadability values of MG-1, MG-2 and MG-3 were 18.6 ± 0.42, 15.2 ± 0.36 and 11.8 ± 0.31 g·cm/s, respectively. The decrease in spreadability with increasing polymer concentration may be attributed to the increased viscosity and resistance to flow. MG-2 demonstrated satisfactory spreadability while maintaining adequate gel consistency.

Extrudability

The extrudability values of MG-1, MG-2 and MG-3 were 96.2 ± 1.2%, 94.8 ± 0.9% and 88.5 ± 1.4%, respectively. MG-1 exhibited the highest extrudability due to its lower viscosity, whereas MG-3 required comparatively greater force for extrusion. MG-2 demonstrated favourable extrusion characteristics along with adequate consistency, indicating its suitability for topical application.

Gel Content Uniformity

The silver-content recovery values for MG-1, MG-2 and MG-3 were 96.4 ± 1.1%, 98.2 ± 0.8% and 95.7 ± 1.3%, respectively. MG-2 exhibited the highest recovery, suggesting comparatively consistent incorporation of the silver nanoparticle component. The uniformity of silver distribution should additionally be confirmed through sampling from different locations within the gel batch.

Table 9: Comparative evaluation of different MECH-AgNP gel formulations

Evaluation parameters	**MG-1**	**MG-2**	**MG-3**
Colour	Light brown	Brown	Dark brown
Odour	Characteristic	Characteristic	Characteristic
Appearance	Smooth gel	Smooth, uniform gel	Thick gel
Homogeneity	Good	Excellent	Good
Visible aggregates	Absent	Absent	Absent
pH	5.82 ± 0.04	6.18 ± 0.03	6.42 ± 0.05
Viscosity (cP)	18,500 ± 320	26,400 ± 410	34,800 ± 520
Spreadability (g·cm/s)	18.6 ± 0.42	15.2 ± 0.36	11.8 ± 0.31
Extrudability (%)	96.2 ± 1.2	94.8 ± 0.9	88.5 ± 1.4
Silver content uniformity (%)	96.4 ± 1.1	98.2 ± 0.8	95.7 ± 1.3
Overall evaluation	Acceptable	Optimized	Acceptable

Values are mean ± SD (n = 3).

Stability studies

The optimized MECH-AgNP gel formulation (MG-2) was evaluated under accelerated storage conditions of 40 ± 2°C and 75 ± 5% RH for three months. The formulation retained its brown colour, characteristic odour and homogeneous appearance without visible phase separation or aggregation. Slight changes in pH, viscosity, particle size and zeta potential were observed during storage. Overall, the formulation demonstrated acceptable preliminary physical and physicochemical stability under the storage conditions.

Table 10: Stability evaluation of optimized MECH-AgNP gel (MG-2)

Parameters	**Initial**	**1 Month**	**2 Months**	**3 Months**
Colour	Brown	Brown	Brown	Brown
Appearance	Smooth	Smooth	Smooth	Smooth
Homogeneity	Excellent	Excellent	Good	Good
Phase separation	Absent	Absent	Absent	Absent

pH	6.18	6.16	6.14	6.12
Viscosity (cP)	26400	26180	25920	25650
Particle size (nm)	85.6	87.2	89.4	91.3
Zeta potential (mV)	−32.4	−32.0	−31.5	−30.8

Selection of the optimized MECH-AgNP gel formulation

Based on the combined evaluation of physical appearance, homogeneity, pH, viscosity, spreadability, extrudability and silver content recovery, MG-2 was selected as the optimized formulation.

Table 11: Selection of the optimized MECH-AgNP gel

Parameter	Observation
Carbopol 940	0.75% w/w
Glycerin	5% w/w
MECH-AgNP dispersion	7.5% w/w
pH	6.18 ± 0.03
Viscosity	26,400 ± 410 cP
Spreadability	15.2 ± 0.36 g·cm/s
Extrudability	94.8 ± 0.9%
Silver content recovery	98.2 ± 0.8%

The MG-2 formulation exhibited a favourable balance of viscosity, spreadability, extrudability and silver content recovery compared with MG-1 and MG-3. Therefore, MG-2 was selected for subsequent stability assessment and *in-vivo* pharmacological evaluation.

***In-vivo* Pharmacological Evaluation**

The *in-vivo* study was conducted to assess the safety and anti-inflammatory potential of *Cocculus hirsutus* methanolic extract (MECH) and MECH-mediated silver nanoparticle gel in Wistar albino rats. The evaluation included preliminary dermal tolerability, clinical observations and biochemical analysis.

Preliminary Dermal Tolerability Evaluation

The preliminary dermal tolerability assessment indicated that the MECH-AgNP gel formulations containing 0.5%, 1.0% and 2.0% w/w extract-equivalent concentrations produced no noticeable skin irritation under the study conditions. No visible erythema, oedema, dryness, scaling, excoriation or ulceration was observed. General behaviour, food and water intake and body weight remained within acceptable limits during the observation period. These findings suggested satisfactory preliminary local tolerability of the tested formulations.

***In-vivo* Pharmacological Evaluation**

Induction of Experimental Chronic Dermatitis

Following topical TCNB exposure according to the approved experimental protocol, the induced animals exhibited visible skin alterations, including erythema, oedema, dryness, scaling and increased skin thickness. These changes were more prominent in the disease-control group than in the normal-control group. The observations indicated the development of experimental dermatitis and provided a basis for evaluating the therapeutic effects of MECH and MECH-AgNP gel formulations.

Experimental Design and Treatment Groups

Table 12: Experimental grouping for evaluation of anti-inflammatory activity

Group	Treatment	Animals
I	Normal control (Vehicle only)	6
II	TCNB-induced disease control	6
III	TCNB + blank gel	6
IV	TCNB + Tacrolimus 0.1%	6
V	TCNB + MECH gel 1%	6
VI	TCNB + MECH-AgNP gel 0.5%	6
VII	TCNB + MECH-AgNP gel 1%	6
VIII	TCNB + MECH-AgNP gel 2%	6
Total		48

Clinical assessment of dermatitis

The disease-control group exhibited a higher cumulative dermatitis score, indicating marked skin inflammation. Treatment with tacrolimus and MECH-based formulations reduced the clinical manifestations compared with the disease-control group. Among the nanoparticle gel groups, the 2% formulation demonstrated the greatest reduction in dermatitis severity.

Table 13: Cumulative dermatitis scores following treatment

Group	Cumulative dermatitis score
I – Normal control	0.20 ± 0.10
II – Disease control	11.80 ± 0.75
III – Blank gel	11.20 ± 0.68
IV – Tacrolimus 0.1%	3.10 ± 0.42

V – MECH gel 1%	5.60 ± 0.55
VI – MECH-AgNP gel 0.5%	6.30 ± 0.61
VII – MECH-AgNP gel 1%	4.20 ± 0.48
VIII – MECH-AgNP gel 2%	3.40 ± 0.39

mean ± SD (n = 6). Lower scores indicate less severe dermatitis.

The results suggest that MECH-AgNP gel produced a concentration-dependent improvement in clinical dermatitis manifestations. The 2% formulation demonstrated the greatest improvement among the nanoparticle-treated groups, with an effect approaching that of the tacrolimus-treated group.

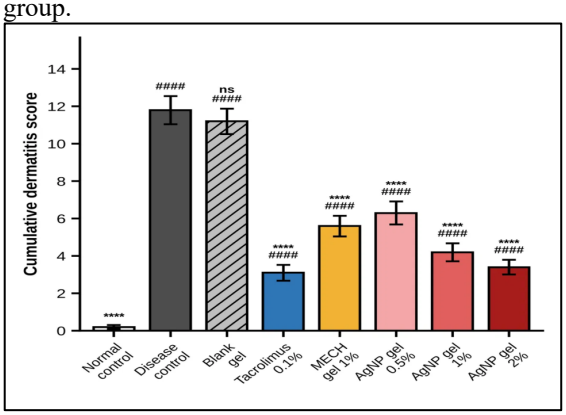

Fig. 6: Cumulative dermatitis scores

Measurement of Skin Thickness

The disease-control group exhibited increased skin thickness compared with the normal-control group. Treatment with MECH gel and MECH-AgNP gel reduced skin thickening, with the 2% nanoparticle gel showing the greatest reduction among the test formulations.

Table 14: Skin thickness following treatment

Group	Skin thickness (mm)
I – Normal control	0.42 ± 0.03
II – Disease control	1.18 ± 0.08
III – Blank gel	1.12 ± 0.07
IV – Tacrolimus 0.1%	0.56 ± 0.04
V – MECH gel 1%	0.72 ± 0.05
VI – MECH-AgNP gel 0.5%	0.79 ± 0.06
VII – MECH-AgNP gel 1%	0.65 ± 0.04
VIII – MECH-AgNP gel 2%	0.59 ± 0.04

mean ± SD (n = 6).

The reduction in skin thickness suggests an improvement in inflammation-associated skin changes. The higher-concentration MECH-AgNP gel demonstrated comparatively better activity than the lower-concentration formulation.

Biochemical Assessment of Inflammatory Markers

The disease-control group exhibited increased inflammatory mediator levels, particularly TNF-α, IL-6 and IL-1β. Treatment with MECH and MECH-AgNP gel reduced these inflammatory markers. The 2% MECH-AgNP gel showed the greatest reduction among the test formulations.

Table 15: Inflammatory cytokine levels in skin tissue

Group	TNF-α (pg/mg protein)	IL-6 (pg/mg protein)	IL-1β (pg/mg protein)
I – Normal control	22.5 ± 2.1	18.4 ± 1.8	12.6 ± 1.2
II – Disease control	86.4 ± 5.6	72.8 ± 4.9	58.2 ± 4.1
III – Blank gel	81.2 ± 5.2	68.5 ± 4.5	54.6 ± 3.8
IV – Tacrolimus	30.2 ± 2.8	26.4 ± 2.2	19.8 ± 1.7
V – MECH gel 1%	44.8 ± 3.6	39.5 ± 3.1	31.6 ± 2.6
VI – MECH-AgNP gel 0.5%	52.6 ± 4.1	45.8 ± 3.5	36.4 ± 2.9
VII – MECH-AgNP gel 1%	37.5 ± 3.0	32.6 ± 2.7	25.4 ± 2.1
VIII – MECH-AgNP gel 2%	32.8 ± 2.7	28.5 ± 2.4	21.6 ± 1.9

mean ± SD (n = 6).

The reduction in inflammatory cytokine concentrations suggests that MECH-AgNP gel may exert anti-inflammatory effects by modulating inflammatory mediator production.

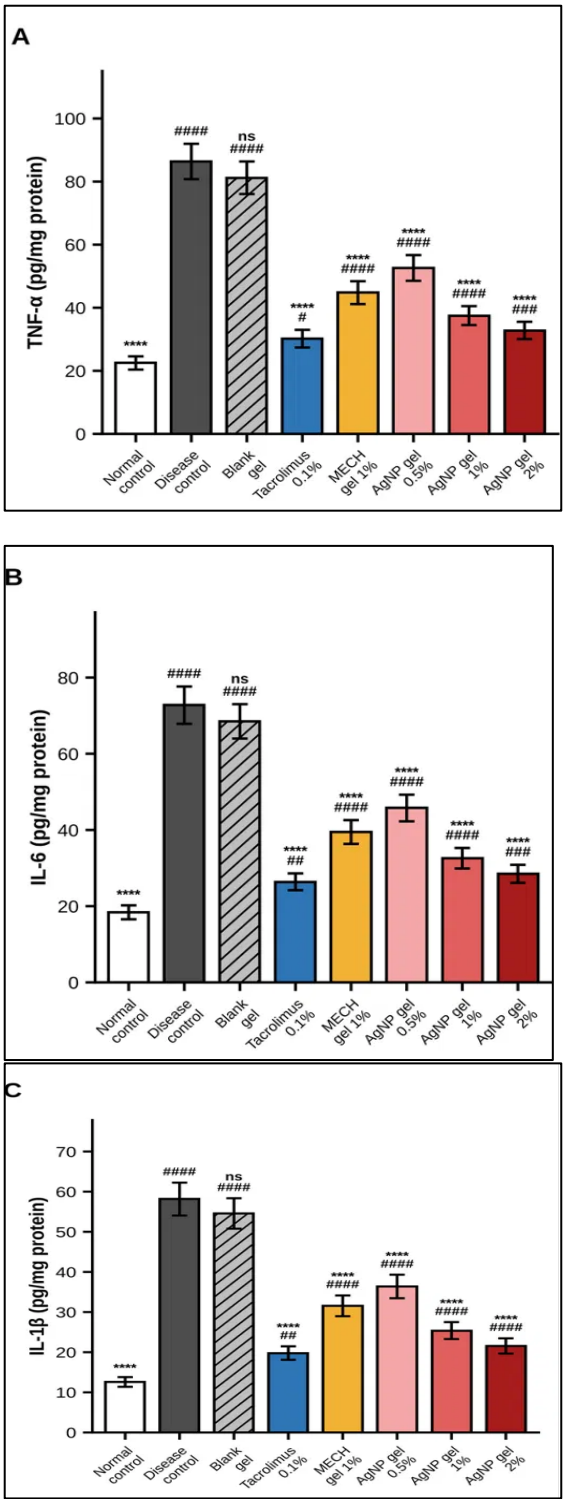

Fig. 7: Biochemical Assessment of Inflammatory Markers (TNF-α, IL-6 and IL-1β)

Assessment of oxidative stress markers

The disease-control group demonstrated increased MDA levels and reduced GSH, SOD and CAT activities compared with the normal-control group. Treatment with MECH-based formulations improved the oxidative stress profile. The 2% MECH-AgNP gel exhibited the most favorable response among the nanoparticle-treated groups.

Table 16: Oxidative stress and antioxidant parameters

Group	MDA (nmol/ mg protein)	GSH (µmol/ mg protein)	SOD (U/mg protei n)	CAT (U/mg protei n)
I – Normal control	1.85 ± 0.16	8.20 ± 0.54	7.80 ± 0.48	5.60 ± 0.38
II – Disease control	6.82 ± 0.48	3.10 ± 0.29	2.85 ± 0.24	2.10 ± 0.18
III – Blank gel	6.45 ± 0.43	3.35 ± 0.31	3.05 ± 0.26	2.25 ± 0.19
IV – Tacrolimus	2.45 ± 0.21	7.15 ± 0.48	6.85 ± 0.42	4.95 ± 0.34
V – MECH gel 1%	3.65 ± 0.32	6.10 ± 0.42	5.65 ± 0.38	4.10 ± 0.29
VI – MECH-AgNP gel 0.5%	4.20 ± 0.36	5.45 ± 0.38	5.10 ± 0.35	3.75 ± 0.27
VII – MECH-AgNP gel 1%	3.10 ± 0.27	6.65 ± 0.45	6.20 ± 0.40	4.55 ± 0.32
VIII – MECH-AgNP gel 2%	2.75 ± 0.23	6.95 ± 0.47	6.55 ± 0.43	4.80 ± 0.33

mean ± SD (n = 6).

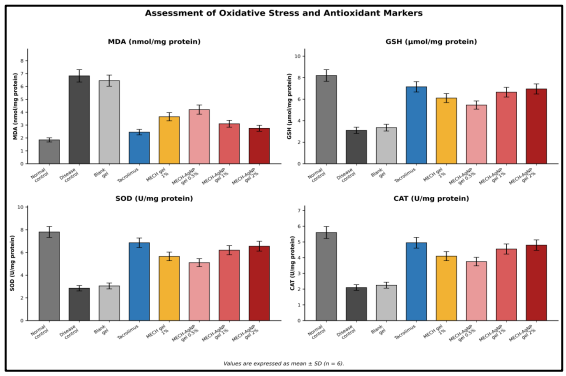

Fig. 8: Oxidative stress and antioxidant parameters

The reduction in MDA levels and improvement in GSH, SOD and CAT activities indicate a possible improvement in tissue oxidative balance following treatment. The results suggest that the antioxidant properties of MECH, together with its nanoparticle-based delivery, may contribute to the observed pharmacological response.

CONCLUSION:

The present investigation explored the development of *Cocculus hirsutus* extract-mediated silver nanoparticles and their incorporation into a topical gel for the management of chronic inflammatory skin conditions. The methanolic extract was selected for nanoparticle synthesis based on its phytochemical characteristics, while physicochemical evaluation provided a basis for assessing nanoparticle formation and formulation suitability. Incorporation of the nanoparticle dispersion into a Carbopol 940-based gel offered a topical delivery approach for further pharmacological investigation. The oxidative stress assessment indicated a treatment-associated improvement in the antioxidant profile, characterized by reduced malondialdehyde (MDA) levels and increased glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities compared with the disease-control condition. Among the tested nanoparticle gel concentrations, the 2% MECH-AgNP gel demonstrated the most favorable overall antioxidant response. These observations suggest that the combination of *C. hirsutus* phytoconstituents and silver nanoparticle-based delivery may contribute to the modulation of oxidative disturbances associated with chronic skin inflammation. Overall, the findings support further investigation of MECH-mediated silver nanoparticle gel as a potential topical formulation. Nevertheless, comprehensive assessment of inflammatory biomarkers, dermal compatibility, and silver nanoparticle-related toxicity is essential to establish its therapeutic efficacy and safety. Further investigations are also required to clarify the underlying mechanisms and determine its suitability for future clinical development.

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AUTHORS CONTRIBUTIONS:

All authors have contributed equally.

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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