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Topical Microgel Formulation Containing Tectona Grandis Bark Extract and Evaluation to Treat Arthritis in an Animal Model

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Abstract
This study aimed to develop and evaluate a topical microgel formulation containing Tectona grandis bark extract for its anti-arthritic and anti-inflammatory activities in rats. Twelve topical gel formulations were prepared using Carbopol 934 (F1-F6) and Carbopol 940 (F7-F12) as gelling agents. These formulations were rigorously evaluated for physicochemical parameters, including net content, physical appearance, viscosity, extrudability, pH, spreadability, and in vitro diffusion profile. Primary skin irritation tests were also conducted to ensure the safety of the product. Among the prepared formulations, F4, formulated with 1.5% Carbopol 934 and 2% Tectona grandis bark extract, demonstrated superior organoleptic characteristics and active ingredient release.
The anti-arthritic activity of F4 was assessed in Freund's Complete Adjuvant (FCA)-induced arthritic rats. Key parameters such as body weight, paw volume, haematological profiles (haemoglobin, ESR, RBC, WBC), biochemical markers (SGPT, SGOT, total proteins, creatinine, uric acid, urea nitrogen), and histopathological examination of joint tissues were evaluated over 28 days. Topical application of formulation F4 significantly ($p < 0.01$) reduced paw volume, restored haematological and biochemical parameters towards normal levels, and showed comparable anti-arthritic effects to standard diclofenac topical gel. Furthermore, histopathological analysis confirmed a reduction in inflammation in the treated groups. The in vitro release kinetics of F4 followed zero-order kinetics, indicating a controlled release profile. The skin irritation tests confirmed the non-toxic and safe nature of the developed microgel.
These findings suggest that the topical microgel formulation containing Tectona grandis bark extract, particularly F4, possesses significant anti-arthritic and anti-inflammatory properties, potentially attributed to the presence of Betulin. This formulation presents a promising alternative for the topical management of arthritis and inflammatory disorders.
Keywords: *Tectona grandis, topical microgel, arthritis, anti-inflammatory, Carbopol, FCA-induced arthritis, controlled release.*

INTRODUCTION

Medicinal plants have played a vital role in the evolution of human culture, serving as a primary source of medicine in virtually all civilisations. These plants are a rich reservoir of traditional remedies and have contributed to many modern medical developments. Medicinal plants have been used to treat various health disorders, highlighting their enduring importance in human well-being [1]. Concerns over toxicity and drug resistance associated with modern pharmaceuticals have driven a surge in interest in natural products for healthcare. India is recognised as one of the world's major biodiversity hotspots and boasts numerous distinct plant species. Thus, India is a rich source of potential medicinal compounds. These plants are valuable resources that provide a wide range of applications, including folk remedies, traditional medicine, modern pharmaceuticals, and chemical entities used in synthetic drug development. This study focuses on traditional medicinal plants for primary healthcare as a global trend.

Plants have long served as a vital source of medicine. Research into natural products often seeks to validate medicinal properties by combining existing scientific knowledge with traditional uses. Phytochemicals isolated from plants can then act as templates to optimise molecules in drug development. Notably, in developing countries, several pharmaceuticals are derived from plants and their constituents [2]. Numerous plants have been the subject of phytochemical and pharmacological studies.

Among these, Tectona grandis L. f. (TG), commonly known as teak (English), Sagwan (Hindi), and Saka (Sanskrit), has garnered significant attention. This species, belonging to the Verbenaceae family, is a large deciduous tree capable of reaching heights of 30–40 meters. Mature trees often exhibit fluting and buttresses at their base. The bark is light greyish-brown, noticeably fibrous with shallow and longitudinal fissures. The leaves are large, 30-40 cm by 15-30 cm, shiny, opposite, and elliptic or obovate, acute or acuminate, and have a grey. The lower surface is covered with glandular hairs, and the upper surface of

the leaf is rough but usually globular. The large leaves shed for 3-4 months during the latter half of the dry season. The tree becomes the twigs plain. Small, white, bisexual flowers bloom in large panicles. Small flowers are about 8 mm across, mauve to white. Flowers about 45 cm long are arranged in large, flowering heads; present in the unshaded part of the crown on the topmost branches of trees. Fruits are 1-3 cm in diameter, irregularly round, green coloured with soft pericarps covered with dense felted stellate hairs, and woody like a stone fruit. A drupe is a round, hard, and woody fruit. This has 4 chambers enclosed in an inflated, bladder-like covering that are pale green and turned brown at maturity. 0 to 4 seeds may be present in one fruit. The roots are superficial, not deeper than 50 cm, and roots may extend sideways up to 15 m from the stem [3, 4]. This Tectona grandis tree, found across several South Asian nations, has long been valued for the medicinal properties found in its various parts, including the roots, bark, flowers, wood, and oil. Traditional and ethnopharmacological practices have utilised these components to treat numerous ailments, from common colds and headaches to more complex conditions. Its applications include wound healing, relief from bronchitis and scabies, and use as a laxative and diuretic. The plant has also been explored for its potential anti-constipating, antidiabetic, anti-inflammatory, antioxidant, and lipid-regulating effects [4]. The plant's primary constituents exhibit several pharmacological activities, including antibacterial, antioxidant, antifungal, anti-inflammatory, antipyretic, analgesic, antidiuretic, and hypoglycemic effects. Specifically, the flowers of Tectona grandis (teak) have been traditionally used to treat bronchitis, biliousness, and urinary discharge. Both the flowers and seeds are employed as diuretics. The wood is reported to have expectorant, anti-inflammatory, anti-bilious, and anthelmintic properties. The bark acts as a potent astringent that is effectively used for bronchitis, arthritis, and inflammation treatment. The root is used for anuria and urinary retention, while the nut oil is used topically to treat scabies and other skin diseases, and is also believed to promote hair growth [6]. Recent phytochemical and pharmacological research has uncovered effective treatments for certain diseases that have eluded the synthetic drug industry. This exploration of pharmacologically and biologically active agents derived from natural sources, such as plant extracts, has resulted in the discovery of numerous valuable pharmaceuticals that play an important role in treating human diseases [7].

Phytochemical constituents

A study on the Most bioactive extract phytochemical from Tectona grandis Linn. led to the isolation of two new nor lignans, tectonoelin A and tectonoelin B compounds. The bioactive fractions of teak have seven apocarotenoids, two of which have been isolated for the first time as natural products named tectoionols A and tectoionols B [8, 9, 10]. Phenolic compounds such as phenolic acids, flavonoids, and tannins are vital plant metabolites. These are responsible for several pharmacological activities of Tectona grandis extract. The four phenolic compounds isolated from T. grandis are TG1, TG2, TG3, and TG4. These are Gallic acid [11] and ellagic acid [12] (phenolic acids), rutin [13] and quercetin [14] (flavonoids) from the methanol extract of T. grandis Linn. The presence of these constituents of teak contributes to the activities by their antioxidant, anti-inflammatory, analgesic, and antimicrobial activities [15].

Pharmacological activities

The global use of herbal products has grown significantly in recent years, reflecting the long-standing importance of plants as sources of medicinal compounds. However, elucidating the complex molecular interactions and mechanisms of action of herbal extracts and their bioactive constituents remains a significant challenge for researchers. This study provides a brief overview of the diverse pharmacological activities of Tectona grandis Linn.

1. **Antioxidant activity:** The antioxidant activity of leaf, bark, and wood extracts (hexane, chloroform, ethyl acetate, and methanol) was evaluated using DPPH and ABTS+ free radical scavenging assays. The ethyl acetate extract of wood exhibited the highest activity, inhibiting 98.6% of DPPH and ABTS+ free radicals. This demonstrates the potential of T. grandis Linn. as an antioxidant, which has also been shown in studies using crude ethanol extracts and evaluating H2O2 scavenging activity, DPPH, and FRAP assays [16]. To assess the potential of T. grandis Linn. leaf extracts for food and medicinal uses, a separate study evaluated their antioxidant activity using four in vitro assays: total phenolic content, reducing power, superoxide radical scavenging activity, and inhibition of H_2O_2-induced erythrocyte hemolysis [17]. An in vitro study investigated the potential of 17 common Indian medicinal plant extracts to regulate nitric oxide (NO) levels, using sodium nitroprusside as an NO donor. Of all the extracts tested, Tectona grandis Linn. (teak) exhibited the most promising NO scavenging activity [18].

2. **Anti-inflammatory activity:** Oral administration of ethanolic and aqueous extracts of Tectona grandis Linn. (teak) stem bark to rats demonstrated significant, dose-dependent analgesic and anti-inflammatory

activity ($p<0.001$) at doses of 100, 300, and 500 mg/kg. At 500 mg/kg, both extracts exhibited significant activity within 15 minutes, lasting up to 120 minutes. Lower doses showed significant activity after 30 minutes, which decreased gradually after 60 minutes. Both extracts demonstrated anti-inflammatory effects at all tested doses. Notably, the aqueous extract exhibited greater anti-inflammatory potential than indomethacin, while the ethanolic extract's activity was comparable. At 500 mg/kg, the aqueous extract showed greater activity than both paracetamol (analgesic standard) and indomethacin (anti-inflammatory standard) ($p<0.001$), an effect less pronounced with the ethanolic extract. Furthermore, the aqueous extract (200 and 400 mg/kg, orally) significantly reduced carrageenan-induced paw oedema compared to controls ($p<0.001$), indicating its anti-oedematogenic effect. This model is a relevant test for anti-inflammatory agents acting on mediators of acute inflammation [19].

3. Antiarthritic activity: Anti-arthritic activity was assessed by measuring proteinase enzyme inhibition. The pooled fraction BVLC-2 exhibited concentration-dependent inhibition (24-71%) at 200-1000 µg/ml concentrations. However, its IC50 value (659.24 µg/ml) indicates lower activity than the standard drug, acetylsalicylic acid (IC50 322.61 µg/ml), tested at the same concentrations. The control showed no proteinase inhibition. Due to limited quantities, the four major semi-pure isolates derived from this fraction could not be compared. Nevertheless, fraction BVLC-2, demonstrating 50% of the anti-arthritic potency of the standard drug, warrants further investigation to identify its bioactive constituents. Proteinases are implicated in arthritis, contributing to tissue damage by degrading the collagen and proteoglycan matrix of bone and cartilage. The presence of flavonoid glycosides in Tectona grandis Linn. (teak) study suggests a potential contribution of these phytochemicals to its anti-arthritic activity [20]**.**

4. Wound healing activity: The study investigated the wound-healing effects of a hydrochloric extract of Tectona grandis Linn. (teak) in rats, comparing its efficacy to the known healing agent Aloe vera. Using excision, incision, burn, and dead space wound models, the extract was tested in a suitable gel formulation via cellophane membrane penetration. In excision and burn wound models, topical application of Tectona grandis Linn. (teak) leaf extract significantly reduced the epithelisation period and promoted wound contraction by 50%. The extract also pointedly increased breaking strength in the incision wound model. In the dead space wound model, oral administration of the Tectona grandis Linn. (teak) leaf extract significantly increased breaking strength, dry weight, and hydroxyproline content of granulation tissue. These results demonstrate that topical (5% and 10% gel) and oral (250 and 500 mg/kg body weight) administration of Tectona grandis Linn. (teak) leaf extract promotes wound healing [21]**.**

5. Immunomodulatory activity: An aqueous Tectona grandis Linn. (teak) bark extract, containing flavonoids, tannins, and phenolic compounds, demonstrated significant immunomodulatory activity at a dose of 100 mg/kg in delayed-type hypersensitivity, cyclophosphamide-induced myelosuppression, and neutrophil adhesion tests. These constituents likely contribute to the observed immunomodulation. Therefore, this aqueous bark extract may be a potential immunoadjuvant for various therapies [23].

This plant possesses a variety of pharmacologically active constituents, exhibiting antibacterial, antioxidant, antifungal, anti-inflammatory, antipyretic, analgesic, antidiuretic, and hypoglycemic properties. Specifically, Tectona grandis flowers treat bronchitis, biliousness, and urinary discharge. Both the flowers and seeds act as diuretics. The wood is an expectorant with anti-inflammatory, anti-bilious, and anthelmintic effects. The bark, a potent astringent, is also used to treat bronchitis. The root is employed for anuria and urinary retention, while the nut oil is for scabies and other skin diseases and to promote hair growth [24]. Recent phytochemical and pharmacological research has successfully addressed some diseases that synthetic drugs have been unable to treat. Consequently, pharmacologically and biologically active agents in natural sources like plant extracts have uncovered numerous valuable drugs crucial for treating human illnesses [25]. The inflammation activity of Tectona grandis Linn. (teak) bark extract was studied based on the pharmacological review of the Tectona grandis Linn plant. Inflammation, a condition often linked to pain, involves increased vascular permeability, protein denaturation, and membrane alteration. Managing inflammation-related diseases poses a significant challenge in rural communities, where people frequently turn to alternative remedies like medicinal plants [25]. Therefore, the present study was designed to evaluate the in vitro anti-inflammatory and antiarthritic activity of Tectona grandis Linn bark extract.

MATERIALS AND METHODS

Materials

The mature and fresh barks of Tectona grandis were collected in July 2023 from Kaleshar National Park, Hathnikund, Yamuna Nagar, Haryana, India. The barks of Tectona grandis were identified by Dr Sunita Garg, Chief Scientist, Head RHMD, CSIR-NIScPR, Raw Material Herbarium and Museum, Delhi, India authentication number NIScPR/RHMD.Consult/2023/4556-57 dated 04/08/2023. Freund's complete adjuvant (FCA), diclofenac sodium, triethanolamine, propylene glycol, and disodium edetate were procured from Sigma-Aldrich USA. Carbopol 934 and Carbopol 940 were procured from Loba Chemie Pvt. Ltd., Mumbai.

Preparation of Tectona grandis serrata extracts

The earthy and residual materials were carefully removed from the bark of Tectona grandis and then dried under the shed. Dried barks were milled to form a coarse powder, divided into three parts, and extracted using petroleum ether (40-60 °C), ethanol, and distilled water in a Soxhlet apparatus for 72 hrs separately. All three extracts were filtered and concentrated under reduced pressure in a rotary evaporator (ILMA Germany, Model Number: RN 10). Concentrates were stored at 4 to 8 degrees Celsius for further use.

Active constituent in the extract estimation

1 g of each extract was transferred into a 50 mL volumetric flask separately. Methanol was added to make a solution of active constituents. Sufficient volume of methanol was added to make the volume. The solution was filtered through Whatman filter paper, and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with methanol. The content of active constituents was estimated spectrophotometrically by using a standard curve plotted at 275 nm

Animal

Healthy female Wistar rats aged between 2 and 3 months and weighing between 150 to 200 g were selected from the central animal house of Animal House, AVIPS, Shobhit University, Gangoh, Saharanpur, Uttar Pradesh. All animals were housed in an animal room under normal conditions at a temperature of 24±1^{0}C °C for 12 hours with light and dark cycles, maintaining humidity 55±5%. The rats were housed individually in polypropylene cages containing sterile paddy husk bedding and free access to food and water ad libitum. The experiments were designed and conducted as per ethical norms approved by the Committee for Control and Supervision on Experiments on Animals (CPSCEA) and the Institutional Animal Ethical Committee through proposal number IAEC-AVIPS/2024/V/0009 (PCL-D) dated 13/05/2024.

Topical gel formulation Base Preparation

1.5 g Carbopol 934 was dissolved slowly with constant stirring in 60 mL purified water for about 60 minutes to avoid agglomeration. 0.005 g Disodium edetate and 1.5 g triethanolamine were dissolved in 10 mL of purified water separately with continuous stirring for 10 minutes. 5 g of propylene glycol was added and mixed in 12 mL of purified water with constant stirring for 10 minutes. Disodium edetate-triethanolamine solution was added to the Carbopol 934 solution. The pH of the solution was adjusted to 7.4, and the solution was stirred for 10 min. Propylene glycol solution was added to Carbopol solution with continuous stirring for 10 min till a clear, consistent topical gel formulation base was developed. The same procedure was repeated to make a topical gel formulation base using Carbopol 940.

Topical gel formulation Preparation

Twelve topical gel formulations of Tectona grandis bark extract concentrate were prepared using the drug formulations mentioned in the table. Formulations F1 to F6 were made using the topical gel formulation base of Carbopol 934 (1.5 %), and formulations F7 to F12 were made using the topical gel formulation base of Carbopol 940 (1.5 %). The F4 formulation, prepared using Carbopol 934 base topical gel formulation, was evaluated for anti-arthritic and anti-inflammatory activity because it had better organoleptic characteristics.

Basic formula for herb topical gel formulation using Petroleum ether extract		
Fo r	Te ct on	Additives Quantity

		Carbopol-934	Carbopol-940	Tri ethanol amine	Disodium EDTA	Propylene Glycol	P. Water
F-1	0.5 gm	1.5 gm	~	1.5 gm	0.005 gm	5 gm	q.s.
F-2	1 gm	1.5 gm	~	1.5 gm	0.005 gm	5 gm	q.s.
F-3	1.5 gm	1.5 gm	~	1.5 gm	0.005 gm	5 gm	q.s.
F-4	**2 gm**	**1.5 gm**	**~**	**1.5 gm**	**0.005 gm**	**5 gm**	**q.s.**
F-5	2.5 gm	1.5 gm	~	1.5 gm	0.005 gm	5 gm	q.s.
F-6	3 gm	1.5 gm	~	1.5 gm	0.005 gm	5 gm	q.s.
F-7	0.5 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.
F-8	1 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.
F-9	1.5 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.
F-10	2 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.
F-11	2.5 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.
F-12	3 gm	~	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s.

Topical herbal gel formulation: Quality control

Active constituents in topical gel formulation (net content) Estimation

1 g of F 4 formulation was transferred into a 50 mL volumetric flask. Methanol was added to make a solution of active constituents. Sufficient volume of methanol was added to make the volume. The solution was filtered through Whatman filter paper, and 0.1 mL of the filtrate was pipetted out and diluted to 10 mL with methanol. The active constituents were estimated spectrophotometrically using a standard curve plotted at 275 nm (27).

Extrudability

About 20 g of the topical gel formulation, contained in a closed collapsible tube, was pressed decisively at the crimped end. A clamp was applied firmly to block any rollback. The topical gel formulation was extruded after removing the topical gel formulation containing collapsible tube cap. The weight of the extruded topical gel formulation was noted after its weighing.

A closed collapsible tube containing about 20 g of topical gel formulation was pressed firmly at the crimped end, and a clamp was applied to prevent rollback. The cap was removed, and the topical gel formulation was extruded. The amount of the extruded topical gel formulation was collected and weighed. The percentage of the extruded topical gel formulation was calculated (28).

pH measurement

The pH of the topical gel formulation was measured using a digital pH meter. The glass electrode of the digital pH meter was completely dipped into the topical gel formulation to measure the pH of the gel formulation. The pH of the topical gel formulation was measured three times, and the average reading was recorded as the pH of the topical gel formulation. (29)

Physical Appearance and Homogeneity

Visual observation was used to evaluate the physical appearance and homogeneity of the topical gel formulation.

Viscosity

Topical gel formulation viscosity was measured at 20 degrees C using a Brookfield viscometer (S-62, model LVDV-E). During viscosity measurement, the topical gel formulation was under spindle speed rotation at 12 rpm (30)

Spreadability

Two glass slides of standard and uniform size were selected. 100 g of the topical gel formulation was placed over one slide. The topical gel formulation was sandwiched by placing the second slide over the slide containing the topical gel formulation and applying uniform pressure to form a thin layer of topical gel formulation. Excess topical gel formulation from the side wall of the slides was scraped off. Both slides were fixed so that one slide (lower slide) remained in a fixed position, and another slide (upper slide) could slide freely after applying weight. The upper slide was tied with the 20-gm weight, carefully. The time required for the upper slide to travel a 7.5 cm distance and separate from the lower slide was noted. The Spreadability of the topical gel formulation was recorded three times. The average value was recorded as the spreadability of the topical gel formulation.

The two slides in position were fixed to a stand without the slightest disturbance, and only the upper slides slipped off freely by the force of the weight tied to it. A 20 g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separate from the lower slide under the influence of the weight was noted. The experiment was repeated three times, and the mean time was taken for calculation. (31).

A formula to calculate the spreadability of the topical gel formulation

$S = m X l/t$

Where,

- S: Spreadability,
- M: Weight tied to upper slides (20 g),
- L: Length of the glass slide (7.5 cm),
- t- Time required in sec

IN VITRO DIFFUSION PROFILE

In vitro permeation in rat skin

A Franz diffusion cell apparatus containing an open-ended cylindrical tube with a 3.8 cm2 area and 100 mm height, and a total diffusion area of 3.8 cm^2 was used to calculate in-vitro diffusion studies of a topical gel formulation. 100 ml of isotonic Phosphate buffer solution, pH 7.4, was added to the donor compartment of the Franz diffusion cell apparatus. The Phosphate buffer solution acted as the receptor medium. Rat abdominal skin was tied to the diffusion cell (donor cell) so that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell. 1 g of topical gel formulation was applied onto the rat skin and was immersed slightly in 100 mL of receptor medium with continuous stirring. 37±1 ^oC temperature was maintained throughout the procedure. 5 ml of diffusion media was withdrawn at intervals of 60 minutes for 8 hours. Active constituent content was estimated by using a spectrophotometer at 275 nm. A fresh equal volume of diffusion media was added after each withdrawal of diffusion media for estimation purposes. The cumulative per cent release of active constituent was calculated for each time 60 minutes.

Release kinetics

The release pattern of an active constituent from a topical gel formulation was fitted to different mathematical models (32) to calculate the release pattern. Concentration-independent kinetics is zero-order kinetics, while concentration-dependent kinetics is first-order kinetics. The release pattern of active constituents may follow swelling and erosion or simply diffusion. Higuchi's model was used to validate data that ascertained the reaction.

Anti-arthritic activity

The FCA-induced arthritis model in rats was used to study the efficiency of the topical microgel formulation. Healthy female Wistar rats aged between 2 and 3 months and weighing between 150 to 200 g were divided into four groups consisting of six animals. Group 1 is considered as normal, Group 2 as FCA-induced arthritis control model, Group 3 as standard topical application of diclofenac topical gel formulation (Voveran topical gel formulation, purchased from retail pharmacy shop), and Group 4 (treatment group) as topical application of formulation F-4. Body weight of rats, paw volume, haematological parameters (haemoglobin, Erythrocyte Sedimentation Rate, Red Blood Cells count, White Blood Cells count, Eosinophil, and Basophil), SGPT and SGOT, X-ray study, Histopathological study and Evan blue test of all rats in all groups were measured during entire 28 days experiment period at 0, 3^{rd}, 7^{th}, 21^{st} and 28^{th} days.

1. **Measurement of rat body weight and paw volume:** The severity of arthritis was quantified by measuring the hind paw volume using a digital Plethysmometer (VJ Instruments Karanja, Maharashtra).

Paw volume (ml) was measured at 0 days and thereafter at 3, 7, 14, 21, and 28 days of FCA post-inoculation. Data were expressed as the increase in paw volume concerning day 0 paw volume[%] and weight by using the digital weighing machine for animals on 3, 7, 14, 21, and 28, days of FCA post-inoculation.

2. **Haematological parameters:** Ketamine (20 mg/kg) was used to anaesthetise the overnight fasted rats. Blood samples were withdrawn from the retro-orbital sinus, and the collected blood samples were centrifuged for 10 minutes at 1000 rpm. The serum was separated from the hematocrit. Haematological parameters such as haemoglobin (Hb) value, red blood cell (RBC) count, white blood cell (WBC) count and erythrocyte sedimentation rate (ESR) were evaluated (33).

3. **Biochemical Analysis:** SGOT (IU/L), SGPT (IU/L), Total Proteins (g%), Creatinine (mg/dl), Uric acid (µg/ml), and Urea nitrogen mmol/L were evaluated by using separated serum. Biochemical analysis was carried out in an autoanalyser (Photometer 5010 V5+, Robert Riely, Berlin) to carry out biochemical investigations using a Piramal Healthcare Limited reagent kit.

4. **X-ray study:** Radiological observation of rats. Evaluation of arthritis onset. Image of joint destruction.

5. Histopathological investigations: Cervical dislocation was used to sacrifice the animals. The organs like the thymus, spleen, and bone joints of the ankle joint, superficial fats were removed, and the organs were weighed after isolation. The isolated ankle joint was immersed in Cal-Ex Decalcifying solution CSS10-1D (Fischer Scientific, India) for 10 days. Decalcified ankle joints were embedded in paraffin. The microtome was used for section cutting (6 micrometres) of embedded ankle joints in paraffin. The thin sections were mounted on microscope slides and stained with Harris hematoxylin and Eosin (34). Histopathological changes in the ankle joints of rats were examined under a microscope, and digital images were captured.

RESULTS AND DISCUSSION

The topical gel formulation has several advantages over other topical formulations due to its following characteristics: high viscosity, more residence time on the skin, high occlusive properties to improve the moisturising effect on flaky skin, high bioadhesive properties, less irritation effect, easy to apply, does not affected by the active constituents' water solubility property, and better release of active constituents to be available at the site of application (35).

Many studies have indicated that Tectona grandis bark containing triterpenes such as Betulin possesses anti-oxidant, anti-inflammatory, and anti-arthritic activities. (17, 18). Therefore, Boswellia serrata bark extract concentrate containing a topical gel formulation was designed to deliver active constituents at the application site in the arthritis treatment.

The polymers Carbopol 934 and Carbopol 940 were selected as topical gel formulation agents to develop a topical gel formulation. Carbopol 934 and Carbopol 940 were used as topical gel formulation agents in the formulation because they have good biodegradable, bio-adhesive, and biocompatible properties. They are also non-irritant to the skin and not absorbed into the body's systemic system through the skin. 0.5 to 2.5% of Carbopol 934 and Carbopol 940 were used separately to formulate a topical gel formulation base. Quality control tests reveal that topical gel formulations containing Carbopol 934 polymer as a topical gel formulation agent are much better than topical gel formulations formulated with Carbopol 940. However, a formulation containing Carbopol 940 polymer has better spreadability quality than one containing Carbopol 934 polymer. Among these topical gel formulation formulations, 1.5 % of Carbopol 934 and 1.5% of Carbopol 940 containing topical gel formulations were compatible with the requirements of topical gel formulation formulations. Six topical gel formulations using 0.5%, 1 %, 1.5 %, 2 %, 2.5 %, and 3 % ethanol extracts of Tectona grandis bark concentrates using 1.5% Carbopol 934 polymer were formulated and evaluated. Formulation F 4, formulated using Carbopol 934, was selected for further study due to its good organoleptic properties. This selection was also correlated as reported better topical gel formulation property of Carbopol 940 and better-controlled release of active phytoconstituents from the topical gel formulation (36). Therefore, in vitro diffusion studies were carried out only for the topical gel formulation preparations F4 formulated using Carbopol 934. Propylene glycol is reported to be the best permeation enhancer (37). Disodium edetate and triethanolamine were used to adjust the pH of the formulation.

Formulated a topical gel formulation. Quality control tests

Twelve topical gel formulations, F-1 to F-12, were formulated using Carbopol polymers 934 and 940, and formulation F-4 was selected and evaluated for their physical appearances, Viscosity, Spreadability, extrudability, pH, and in vitro diffusion profile. F-4 formulation had good consistency, and appearance

and a homogeneous preparation with pH 7.63, viscosity of 0.39 poise, spread-ability 64.21 gm Sec, net wet content 104.5% w/w, extrudability of more than 90% that comes under the excellent category, and physical appearance of Dark green, smooth, homogenous, translucent and does not cause skin irritation. Five readings of each parameter were noted, and their average is tabulated in the table.

Formulation	% Conc. Carbopol 934.	pH	Viscosity in Poise	Spread-ability in gm second	Extrudability	Net Content % w/w	Physical Appearance
F-1	0.5	7.56	0.3848	31.99	Good	100	Dark green, smooth, translucent
F-2	1.0	7.58	0.3858	44.98	Excellent	104	Dark green, Translucent Smooth, & Homogenous,
F-3	1.5	7.59	0.3871	56.41	Good	105	Dark green, Translucent Smooth, & Homogenous,
F-4	2.0	7.63	0.3900	64.21	Excellent	106	Dark green, Translucent Smooth, & Homogenous,
F-5	2.5	7.82	0.3912	70.97	Excellent	101	Dark green, Translucent, Smooth, & homogeneous,
F-6	3.0	7.89	0.3920	75.78	Excellent	99.99	Dark green, Translucent, Smooth, & homogeneous,

In vitro diffusion profile and release kinetics

The membrane used in the in-vitro diffusion study of the topical gel formulation had a pH range from 5 to 7.7, and the isotonic phosphate buffer saline had a pH of 7.4. Almost 100% release of active ingredients was observed within 6 hours from all formulations, F-1 to F-6. The release pattern of active constituents correlated with the market preparation of diclofenac topical gel formulation. F-4 had a better release property of 98.8% among all formulations. F-4 formulation shows zero-order kinetics preferred in control release dosage forms. F-4 was selected for in vivo studies. In vitro, the release kinetics of topical gel formulations F-1 to F-6 with Carbopol 934 polymer were recorded in the table.

Formulation	Zero Order R-1	Zero Order R-2	Higuchi diffusion model R-3	Model Observed
F-1	0.970	0.902	>1	Zero Order
F-2	0.964	0.941	0.912	Zero Order
F-3	0.913	0.929	>1	First Order
F-4	0.999	0.911	>1	Zero Order
F-5	0.897	0.899	0.899	Higuchi
F-6	0.931	0.901	0.901	Higuchi

Skin irritation test

The skin irritant effect of the formulated topical gel formulation base of both Carbopol polymers 934 and Carbopol polymer 940 was evaluated. No erythema or oedema was observed for all the formulations (Table 4). This indicates that the topical gel formulation bases were safe for the skin.

Days	Rabbit				Average
	1	2	3	Control	
Day-1	0	0	0	0	0
Day-2	0	0	0	0	0
Day-3	0	0	0	0	0
Day-4	0	0	0	0	0

Body weight

Rats' average body weight gained/reduced was observed after induction of arthritis and recorded as mentioned in the table. Reduction in body weight was observed in arthritic rats under the control group,

while gain in body weight was observed in all rats under the diclofenac topical gel formulation treatment group and the Formulation F-4 treatment group.

Body Weight in Days						
Treatment Groups (n=6) (Dose mg/kg)	0 day	3rd day	7th day	14th day	21st day	28th day
Normal	**158.9±7.313**	**160.5±4.786**	**161.2±4.785**	**161.6±4.477**	**164.9±4.241**	**165.9±4.570**
Control	172.0±12.46	171.1±12.34	168.4±13.03	167.8±11.91	166.2±10.70	165.3±9.4220 c
Diclofenac topical gel formulation	167.8±5.095	168.7±4.078	168.9±4.153	169.2±4.394	169.8±4.688	171.3±4.055
1% Tectona grandis **bark extract topical gel formulation**	161.4±7.688	162.1±7.973	163.0±8.326	164.6±7.917	164.7±7.945	165.9±7.943

Paw volume (table 6)

The changes in rats' paw volume were recorded on 0, 3rd, 7th, 14th, 21st, and 28th days after diclofenac sodium topical gel formulation application and the topical gel formulation formulation-F4. Reading was recorded in the table. An increase in paw volume was observed in rats under the arthritic control group, showing arthritis development. The reduction in rat paw volume was observed in the treatment group under the diclofenac sodium topical gel formulation and the topical gel formulation F-4 significantly (P less than 0.01). The arthritis severity was assessed visually using a visual scoring arthritic system. (38), and scores were recorded in the table. The visual scoring arthritic system indicates that the FCA-induced arthritis pain was significantly decreased in rats in the treatment group under diclofenac sodium topical gel formulation and the topical gel formulation-F4. flexion pain test score, mobility score, and stance score were observed in all rats under the treatment groups and were compared with rats under the arthritic control group. Results observed support the antiarthritic activity of the topical gel formulation F-4 and were comparable to the marketed diclofenac sodium topical gel.

Rat Paw Oedema volume (ml)						
Treatment Groups (n=6) (Dose mg/kg)	0 day	3rd day	7th day	14th day	21st day	28th day
Normal	0.043±0.020	0.52±0.071	0.44±0.077	0.07±0.034	0.046±0.023	0.048±0.024
Control	0.030±0.018	2.402±0.144 a	2.369±0.119 a	2.419±0.119 a	2.420±0.116 a	2.285±0.095 a
Diclofenac gel	0.019±0.009	1.566±0.085 a	1.668±0.073 a	1.651±0.078 b	1.034±0.095 a	0.899±0.093 a
1% Tectona grandis **bark extract gel**	0.038±0.016	1.41±0.072b	**1.599±0.130 a**	**1.568±0.160 a**	**1.009±0.159 a**	**0.806±0.087 a**

This alteration of arthritic test scores supports the anti-arthritic activity of the topical gel formulation F4. Among the formulations F1 to F6, the F4 formulation was selected for the anti-arthritic study as the results of quality control evaluation of formulation were found to be good, and the in vitro release characteristics of the prepared topical gel formulation F4 were quite encouraging and in agreement with the marketed diclofenac sodium topical gel formulation.

FCA-induced arthritis in rats develops clinical and pathological changes comparable to clinical and pathological changes observed in human rheumatoid arthritis (39). Therefore, FCA-induced arthritis in rats is the most acceptable and widely used model to study the antiarthritic effects of API. The rats are unique in developing polyarthritis after FCA treatment. It is noted that FCA-induced polyarthritis in rats is associated with an immune-mediated inflammatory reaction (40). Arthritic scores reduction, thymus weight reduction, and spleen weight reduction in all rats under treatment groups support the antiarthritic activity of the topical gel formulation F-4. **Table 7**

Groups	**Pain test**		**Mobility Score**	**Stance score**
	Extension	**Flexion**		
Control	**9.4 ± 0.35**	**8.31 ± 0.30**	**1.33 ± 0.21**	**1.50 ± 0.22**
Diclofenac gel	**5.14 ± 0.15$_d$**	**4.7 ± 0.19 $_d$**	**2.66 ± 0.21d**	**2.31 ± 0.20$_d$**
1% w/w Tectona grandis **bark extract gel**	**4.67 ± 0.20$_d$**	**3.65 ± 0.31$_d$**	**3.16 ± 0.21d**	**2.79 ± 0.14$_d$**

Hematological parameters (table)

Reduction in the level of erythropoietin occurs during arthritis. This results in premature destruction of RBCs, which leads to a decrease in RBC count and haemoglobin level. An increase in WBC level has also been reported in arthritis.

A decrease in WBC count, ESR, and an increase in Hb and RBC count were observed in rats under the treatment group of diclofenac sodium gel and topical gel formulation F-4 compared to the control group. This supports the antiarthritic effect of the topical gel formulation F-4.

Haematology profile				
Treatment Groups (n=6) (Dose mg/kg)	**Haemoglobin g/dl**	**ESR mm/h**	**WBC ×103/mm3**	**RBC ×106/mm3**
Normal	14.14±0.603	3.816±0.141	10.38±0.435	7.015±0.351
Control	11.58±0.292c	12.42±0.86a	11.98±0.632	6.867±0.563
Diclofenac gel	13.49±0.184a	5.749±0.518a	10.64±0.488	6.799±0.138
1% Tectona grandis **bark extract gel**	13.44±0.262a	5.782±0.308a	10.67±0.562	6.787±0.2230

Biochemical parameters (Table 9)

Urea and uric acid concentration was observed in the diclofenac sodium gel and the topical gel formulation F-4-treated groups and the arthritic control group. A decrease in urea and uric acid concentration was detected in diclofenac sodium gel and topical gel formulation F-4-treated groups, compared to the arthritic control group.

Biochemistry parameters						
Treatment Groups (n=6) (Dose mg/kg)	**SGOT (IU/L)**	**SGPT (IU/L)**	**Total Proteins (g%)**	**Creatinine (mg/dl)**	**Uric acid (mg/dl)**	**Urea nitrogen**
Normal	57.37±1.200	62.14±3.601	6.86±0.2379	0.742±0.620	5.36±0.04	42.20±2.52
Control	56.59±0.972	65.40±4.44	6.75±0.318	0.784±0.69	5.61±0.04	44.21±2.52
Diclofenac gel	56.93±3.99	62.73±1.926	6.76±0.369	0.79±0.740	5.39±0.221	42.90±2.72
1% Tectona grandis bark extract gel	56.25±1.619	61.72±3.129	6.69±0.186	0.76±0.013	5.6±0.222	42.15±2.44

Histopathological examination

Histological examination of rats in the normal specimen group, arthritis control specimen group, diclofenac sodium topical gel-treated groups, and topical gel formulation F4-treated arthritic rats was carried out. Rats' specimens under the normal specimen group had normal joint space, normal adjacent soft tissue, synovium, and cartilage. Rats' specimens in the arthritis control group had dense inflammation in the soft tissue around the joint. The rats' specimens in the diclofenac sodium topical gel-treated groups had a reduction in inflammation. The rat's specimen in the topical gel formulation F4 had inflammation reduction.

CONCLUSION

Anti-arthritic activity of the developed topical herbal gel formulation may be due to the presence of Betulin in Tectona grandis bark extract. The formulation developed using 1.5% Carbopol 934 was found to be a promising topical gel formulation to treat arthritis and inflammatory disorders.

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