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Topical microgel Formulation containing Boswellia serrata 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 Boswellia serrata bark extract for its anti-arthritic and anti-inflammatory activities in rats. Twelve topical gel formulations were prepared using 1.5% Carbopol 934 (F1-F6) and Carbopol 940 (F7-F12) as gelling agents. These formulations were rigorously evaluated for physical appearance, homogeneity, viscosity, extrudability, pH, spreadability, in vitro diffusion profile, and primary skin irritation. Formulation F4, prepared with Carbopol 934, demonstrated superior organoleptic characteristics and active ingredient release.

The anti-arthritic activity of F4 was assessed using the Freund's Complete Adjuvant (FCA)-induced arthritis model in Wistar rats. Parameters including body weight, paw volume, haematological profiles (haemoglobin, ESR, RBC, WBC), biochemical markers (SGPT, SGOT, total proteins, creatinine, uric acid, urea nitrogen), and histopathological examination were evaluated over 28 days.

The formulated gels were homogeneous, stable, and non-irritating to the skin, showing no erythema or oedema. Topical application of F4 significantly reduced paw volume ($p<0.001$) in arthritic rats compared to the diseased control group. Furthermore, F4 helped restore normal haematological and biochemical parameters, reduced spleen and thymus weights, and showed improvements in joint histopathology, comparable to the standard diclofenac gel. The in vitro release kinetics of F4 followed a zero-order model, indicating controlled release. These findings suggest that the developed Boswellia serrata topical microgel formulation (F4) possesses significant anti-arthritic and anti-inflammatory properties, offering a promising alternative for arthritis management with minimal systemic side effects.

Keywords: *Boswellia serrata, topical microgel, arthritis, anti-inflammatory, Carbopol, FCA-induced arthritis, controlled release*

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1. INTRODUCTION

Arthritis is a joint inflammatory disease that mainly damages the joint cartilage and surrounding tissues. This develops joint fibrosis, fracture, joint degradation, defects, along damage to the entire bony articular surfaces. This leads to chronic pain and loss of mobility in individuals suffering from arthritis. (1)

The primary focus of arthritis management is to reduce pain using nonsteroidal anti-inflammatory drugs (NSAIDs) and specific cyclooxygenase II (COX-2) inhibitors. These drugs are known to be associated with gastrointestinal, renal, and cardiovascular risks. Thus, there is a need for an effective alternative therapy that can reduce the use of these drugs or complement their use with minimal adverse side effects.

Arthritis is a major joint problem that affects the physical activities of individuals. Arthritis is associated with inflammation in joints that require high concentrations of drugs at the inflammatory sites. A high concentration of drugs at inflammatory sites could be achieved without major systemic side effects of drugs by using topical dosage forms. (2). Most commonly, arthritis is managed by reducing chronic pain in joints. For this purpose, non-steroidal anti-inflammatory drugs (NSAIDs) that act as cyclooxygenase II (COX-2) inhibitors are used. However, these drugs develop gastrointestinal, renal, and cardiovascular problems upon long-term use. (3). Therefore, an effective remedy with minimal side effects is needed for an hour to manage arthritis.

Various plants, as a whole, their parts or extracts, such as Capsicum annuum, Butea monosperma, Piper nigum, Gossypium herbaceum, Curcuma longa, Zingiber officinale, Camellia sinensis, Commiphora mukul, Boswellia serrata, etc, have been

used to manage arthritic pain in the Ayurveda system.,

Boswellia serrata belongs to the family: Burseraceae; Genus: Boswellia, a moderate to large-sized, branched, and deciduous tree that grows in dry and hilly parts of India, such as Gujarat, Rajasthan, Madhya Pradesh, Jharkhand, Andhra Pradesh, and Chhattisgarh in India (4). In the plant kingdom, the family of Burseraceae has 17 genera and 600 species spread across all tropical regions. All about 25 known species belong to the Genus Boswellia, and most of them occur in India, Arabia, and the northeastern coast of Africa. Among them, three species of Boswellia are considered 'true Frankincense' producing trees. These are Boswellia sacra, Boswellia carterii, and Boswellia serrata. [5,6]. Boswellia sacra grows in South Arabia. In the native language, it is known as maghrayt d'sheehaz, and the oleo gum resin produced by it is known as 'luban dhakar'. Boswellia carterii grows in Somalia, and the native language is known as 'moxor', and the oleo gum resin produced by it is known as 'luban dhakar'. Another Somalian species of Boswellia is Boswellia frereana. In the native language, it is called 'jaguar' and the oleo gum resin produced by it is known as 'loban made' or 'maybe' (7). Boswellia serrata grows in India and is also known as 'Indian olibanum', 'Indian frankincense', 'dhup' Shallaki, and 'salai' or 'salai guggul'.

Botanical Classification of Boswellia serrata

Division: Spermatophyta

Sub-division: Angiospermae

Tribe: Rosopsida

Sub-tribe: Rosidae S. lat.

Over-class: Rutanae

Class: Anacardiales

Family: Burseraceae

Genus: Boswellia

Species: serrata

Vernacular names of Boswellia serrata

Sanskrit: Ashvamutri, Kundara, Shallaki, Gajabhakshya.

Hindi: Kundur, Salai

Gujarati: Dhup, Gugali

Bengali: Kundur, Salai

Kannada: Chitta, Guguladhuph

Tamil: Parangi, Saambraani

Malayalam: Parangi, Saambraani

Telugu: Phirangi, Saambraani

English: Indian Olibanum or Indian frankincense

The plant exudate of the genus Boswellia is an oleo gum resin that is collected by the incision made on the trunk of the Boswellia tree, which is stored in a bamboo basket specially made for this purpose. The incision on the Boswellia serrata tree trunk is made in March and April and the exudate is harvested in the summer and autumn seasons. A healthy Boswellia serrata tree can produce good-quality exudate for three consecutive seasons. Trees are allowed to rest for three years after three years of exudate collection. Fresh oleo gum-resin exudate of the Boswellia tree is hot with a pleasant flavour and slightly bitter in taste, semi solid and is stored in a specially made bamboo basket for about one month. The fluid from oleo gum resin flows out during storage, which is known as "ras". The pure oleo gum resin hardens slowly during the storage period and develops colour from golden brown to dark brown/dark greenish brown. The colour developed in the oleo gum resin exudate of the Boswellia serrata tree depends on the locality, season, tree size, wound surface, collection process, and storage condition. The autoxidation, polymerization, and enzymatic reactions play important roles in the colour development of Boswellia serrata tree oleo gum resin exudate. After solidification, the remaining solid part is an amorphous, tear-shaped solid product with an aromatic aroma. The size of the solid product is reduced by using a wooden mallet. The flavour, colour, shape, and size of the final product decide the grade of resin. In the market, four grades of Boswellia plant resin are available as Superfine, Grade I, Grade II, and Grade III. It is most commonly used in manufacturing incense powder and sticks.

The traditional Ayurvedic and Unani texts describe various uses of Boswellia serrata exudates to treat arthritis, diarrhoea, dysentery, ringworm, boils, fevers, skin and blood diseases, cardiovascular diseases, mouth sores, bad throat, bronchitis, asthma, cough, vaginal discharges, hair loss, jaundice, syphilitic diseases, irregular menses, and liver malfunctions. It is

also diaphoretic, astringent, and diuretic, and acts both as an internal and external stimulant. Modern studies also strongly suggest evidence of its effect as an antiarthritic, anti-inflammatory, antihyperlipidemic, antiatherosclerotic, analgesic (pain-reliever), and hepatoprotective [8-12].

The oleo gum resins of the Boswellia serrata tree exudate contain 30-60% resin, 5-10% essential oils, and 30-65% polysaccharides (arabinose, galactose, xylose). Essential oils are soluble in organic solvents, and polysaccharides are soluble in water [13-15]. The Boswellia serrata tree exudate has a pleasant aroma due to the presence of essential oils that develop its commercial importance.

Boswellia serrata exudate is an oleo gum resin, also known as Dhup, Salai, Salai guggal, Indian Frankincense, or Indian Olibanum in the Ayurvedic system of medicine, Salai guggal (16). In the Ayurvedic medicinal system has been used to manage arthritis due to its antioxidant and anti-inflammatory properties. The antioxidant and anti-inflammatory properties of B. serrata Roxb. (Family: Burseraceae) are due to its active constituent Boswellic acids (BAs), a triterpene present in its gum resins. (17,18). Boswellic acid is soluble in ethanol and sparingly soluble in water.

3-acetyl-11-keto-β-boswellic acid (AKBBA), 11-keto-β-boswellic acid (KBBA), β-boswellic acid (BBA), and 3-acetyl β-boswellic acid (ABBA), β-pentacyclic triterpene acids, are major bioactive boswellic acids present in the oleo gum resin of Boswellia serrata (19, 20). Out of four β-pentacyclic triterpene acids present in boswellic acids, 3-acetyl β-boswellic acid (ABBA) is the most potent leukotriene-mediated inflammatory pathways and 5-lipoxygenases (5-LO) activities (21,22,23). Boswellia and its extract have positive pharmacological effects to relieve pain, stiffness, and to improve joint movement (24). B. serrata extracts formulation containing not less than 30% 3-acetyl-11-keto-β-boswellic acid and other β-boswellic acids has been reported to have positive pharmacological effects on controlling arthritis in a preclinical arthritis model (23). In the present study, the clinical effects of the Boswellia serrata bark extract topical gel formulation were evaluated to manage arthritis.

Boswellia serrata has an elimination half-life of 4.5±0.55 h with poor bioavailability. Thus, the preferred alternative to the Boswellia serrata oral dosage form is its topical dosage form. Boswellia serrata exudates extract is an oleo gum resin with high lipophilicity, thus it is difficult to develop a proper topical dosage form with optimum permeability through the topical route. (25). Various studies have confirmed that molecules having intermediate lipophilicity (log P, o/w of 2-3) have permeability through both the lipid and polar microenvironments within the intercellular route [25]. The major drawback of B. serrata topical formulation can be overcome by developing a topical microgel formulation that will enhance the permeability of Boswellia serrata extract bioactive upon topical application.

2. MATERIALS AND METHODS

Materials

The mature and fresh barks of Boswellia serrata were collected in July 2023 from Sariska National Park, Alwar, Rajasthan, India, and were identified by Dr K. Madhava Chetty, PLANT Taxonomist (that: 337), Sri Venkateshwar University, Tirupati, Andhra Pradesh, India. 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 Boswellia serrata extracts

The earthy and residual materials were carefully removed from the barks of Boswellia serrata and then dried under the shed. Dried barks were milled to form a coarse powder, divided into three parts, and extracted using ethanol 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. Ethanol was added to make a solution of the active constituent. A sufficient volume of ethanol 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 ethanol. The content of active constituents was estimated spectrophotometrically by using a standard curve plotted at 275 nm.

Animal

Healthy female Wistar rats with ages between 2 and 3 months and weights 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 inside the animal house under normal conditions at a temperature of 24±1 °C for 12 hours with light and dark cycles, maintaining humidity of 55±5%. The rats were housed individually in polypropylene cages containing sterile paddy husk bedding along with 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 microgel formulation Base Preparation

To avoid agglomeration, 1.5 g Carbopol 934 was dissolved slowly with constant stirring in 60 mL purified water for about 60 minutes. 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 Microgel Formulation Preparation

Twelve topical gel formulations of Boswellia serrata extract concentrate were prepared using the drug formulations mentioned in Table 1. 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 by using Carbopol 934 base topical gel formulation, was evaluated for anti-arthritic activity because it had better organoleptic characteristics.

Basic formula for herb topical gel formulation using *Petroleum ether extract*							
Formulations	Boswellia serrata ***bark powder Extract***	**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 to produce 100 gm.
F-2	1 gm	1.5 gm	--	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-3	1.5 gm	1.5 gm	--	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-4	**2 gm**	**1.5 gm**	**--**	**1.5 gm**	**0.005 gm**	**5 gm**	q.s to produce 100 gm.
F-5	2.5 gm	1.5 gm	--	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-6	3 gm	1.5 gm	--	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-7	0.5 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-8	1 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-9	1.5 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-10	2 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-11	2.5 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.
F-12	3 gm	--	1.5 gm	1.5 gm	0.005 gm	5 gm	q.s to produce 100 gm.

Topical herbal topical microgel 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 the active constituent. 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 (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 the removal of 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 any 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 by 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 by 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. The excess topical gel formulation adhering to the side wall of the slides was scraped off. Both slides were fixed in a manner 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 noted as the spreadability of the topical gel formulation.

The two slides in position were fixed to a stand without the slightest disturbance and in such a way that 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).

Formula to calculate the spreadability of the topical gel formulation

$S = m \times l/t$

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 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 having a pH of 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 also 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 interval.

Release kinetics

The release pattern of an active constituent from formulation 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 to ascertain 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, each consisting of six animals. Group 1 is considered 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, 3rd, 7th, 21st and 28th days.

Measurement of rat body weight and paw volume: The severity of arthritis was quantified by measuring the volume of the hind paw 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[96] and weight by using the digital weighing machine for animals on 3, 7, 14, 21, and 28 days of FCA post-inoculation.

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).

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.

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

Histopathological investigations: Cervical dislocation was used to sacrifice the animals. After isolation of the organs like the thymus, spleen, and bone joints of the ankle joint, superficial fats were removed, and the organs were weighed. 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.

3. RESULTS AND DISCUSSION

The topical microgel formulation has several advantages over other topical formulations due it its characteristics such as 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 triterpenes such as Boswellic acids (BAs) in herbs possess anti-oxidant, anti-inflammatory, and antiarthritic activities. (17, 18). Therefore, Boswellia serrata bark extract concentrate containing a topical gel formulation was designed to deliver an active constituent at the site of application in the treatment of arthritis.

Formulated topical microgel 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, good appearance, and homogeneous preparation with pH 7.63, viscosity 0.39 poise, spreadability 64.21 g Sec, net wet content 104.5% w/w, extrudability of more than 90% which comes under the excellent category, and physical appearance 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	Light brown, smooth, & Translucent
F-2	1.0	7.58	0.3858	44.98	Good	101	Brown, Translucent Smooth, & Homogenous,
F-3	1.5	7.59	0.3871	56.41	Good	103	Dark-green, Translucent Smooth, & Homogenous,
F-4	2.0	7.63	0.3900	64.21	Excellent	105	Dark-green, Translucent Smooth, & Homogenous,
F-5	2.5	7.82	0.3912	61.97	Good	101	Dark-green, Translucent Smooth, & Homogenous,
F-6	3.0	7.89	0.3920	59.78	Good	99.99	Dark-green, Translucent Smooth, & Homogenous,

The polymers Carbopol 934 and Carbopol 940 were selected as topical gel formulation agents to develop a topical microgel formulation. Carbopol 934 and Carbopol 940 were used as topical gel-formulating 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 microgel formulation base. Quality control tests reveal that a topical microgel formulation containing Carbopol 934 polymer as a topical microgel formulating agent is much better than a topical gel formulation formulated with Carbopol 940. However, a formulation containing Carbopol 940 polymer has better spreadability quality than a formulation containing Carbopol 934 polymer. Among these topical gel formulations, 1.5 % of Carbopol 934 and 1.5% of Carbopol 940 containing topical microgel formulations were found to be compatible with the requirements of a topical microgel formulation. Formulation F 4, formulated using Carbopol 934, was selected for further study purposes due to its good organoleptic properties and better microgel quality. This selection was also in correlation with the 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 microgel formulation preparations F4, formulated using Carbopol 934. Propylene glycol has been reported as the best permeation enhancer (37). Disodium edetate and triethanolamine were used in the formulation to adjust the pH of the formulation.

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. Among all these formulations, F-4 had a better release property, which was 98.8%. F-4 formulation shows zero-order kinetics, which is preferred in controlled-release dosage forms. F-4 was selected for in vivo studies. In vitro, release kinetic study of topical gel formulation formulations F-1 to F-6 with Carbopol 934 polymer was recorded in Table 3.

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). This indicates that the topical gel formulation bases were safe to apply to 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	165.4±4.570
Control	161.0±12.46	159.1±12.34	157.4±13.03	151.8±11.91	152.2±10.70	143.3±9.4220c
Diclofenac topical gel formulation	166.2±5.075	160.7±4.078	159.3±4.043	161.2±4.314	164.8±4.598	167.3±4.041
2% Boswellia serrata *bark* extract topical gel formulation	161.4±5.656	159.1±5.973	158.0±5.324	158.9±5.912	162.7±5.915	165.9±7.543

Paw volume

The changes in rats' paw volume were recorded on 0, 3rd, 7th, 14th, 21st, and 28th days after application of diclofenac sodium topical gel formulation 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. Reduction in rat paw volume was observed in the treatment group under diclofenac sodium topical gel formulation and 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 the 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 it was 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	0.052±0.071	0.044±0.077	0.07±0.034	0.046±0.023	0.049±0.024
Control	0.030±0.018	2.492±0.141a	2.369±0.109a	2.419±0.108a	2.420±0.116a	2.295±0.095a
Diclofenac gel	0.019±0.009	1.566±0.084a	1.668±0.075a	1.651±0.077b	1.044±0.094a	0.869±0.092a
2% Boswellia serrata ***bark*** **extract gel**	0.038±0.016	1.41±0.072b	**1.599±0.130a3**	**1.568±0.160a**	**1.009±0.159a**	**0.806±0.087a**

This alteration of arthritic test scores supports the anti-arthritic activity of the topical herbal microgel 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 microgel formulation F4 were quite encouraging and in agreement with the marketed diclofenac sodium topical gel formulation.

Haematological parameters

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±0.598	3.809±0.139	10.38±0.429	7.015±0.349
Control	10.98±0.301c	12.39±0.79a	12.08±0.602	6.199±0.413
Diclofenac gel	13.49±0.184a	5.749±0.518a	10.64±0.488	6.799±0.138
2% Boswellia serrata ***bark*** **extract gel**	13.44±0.262a	5.992±0.308a	10.59±0.562	6.987±0.123

Biochemical parameters (Table 9)

Urea and uric acid concentration was observed in the diclofenac sodium gel, 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 in comparison with 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	56.97±1.210	61.98±3.598	6.66±0.229	0.739±0.619	5.29±0.03	42.18±2.22
Control	55.99±1.002	64.89±4.14	6.79±0.229	0.790±0.41	5.41±0.04	41.91±2.49
Diclofenac gel	57.00±4.01	62.13±2.006	6.72±0.298	0.761±0.548	5.39±0.221	42.90±2.69
2% *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

FCA-induced arthritis in rats develops clinical and pathological changes that are comparable with 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 anti arthritic effects of API. 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.

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. Rats' specimens in the diclofenac sodium topical gel-treated groups had a reduction in inflammation. Rat's specimen in topical gel formulation F4 had a reduction in inflammation.

4. CONCLUSION

Anti-arthritic activity of the developed topical herbal gel formulation may be due to the presence of Betulin in *Boswellia serrata 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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