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Development of A Herbal-Based Hydrogel: A Promising Approach For Inflammatory Disorders

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ABSTRACT

Aim: The present study aimed to develop and evaluate a polyherbal hydrogel formulation incorporating hydro alcoholic extracts of Cordio obliqua, Tinospora cardifolia, Lantana camara, Spharanthus indica, and Grewia abutifolia for its anti-inflammatory, antimicrobial, and anti-arthritic activities. Materials and Methods: Six formulations of polyherbal hydrogel were prepared using Carbopol-940, ethanol, propylene glycol, methyl paraben, EDTA, propyl paraben, and triethanolamine. The formulations were characterized for their physical properties, pH, spreadability, viscosity, and swelling index. Anti-inflammatory activity was assessed via protein denaturation and membrane stabilization assays. Antimicrobial activity was evaluated using the well-diffusion method against E. coli, P. aeruginosa, S. aureus, and B. subtilis. In vivo studies, including acute dermal toxicity and anti-arthritic efficacy, were conducted using Freund's adjuvant and carrageenan-induced paw edema models in rats. Results: The prepared hydrogels were homogenous, translucent, and had a pH range of 6.8-7.3. Formulations PF3 and PF5 exhibited optimal viscosity, swelling index, and spreadability. The in vitro assays demonstrated significant anti-inflammatory activity, with PF5 showing the highest inhibition in protein denaturation and membrane stabilization assays. The hydrogel formulations also exhibited antibacterial activity, particularly against E. coli and S. aureus. In vivo toxicity studies confirmed the gel's safety, with no observable toxic effects. The anti-arthritic evaluation revealed a reduction in paw edema and joint inflammation in treated groups, with PF5 demonstrating superior efficacy compared to the standard diclofenac sodium gel. Conclusion: The formulated polyherbal hydrogel demonstrated promising anti-inflammatory, antimicrobial, and anti-arthritic properties, supporting its potential as a topical therapeutic agent. The results suggest that PF5 is the most effective formulation for further pharmacological and clinical studies.

Keywords: Polyherbal hydrogel, anti-inflammatory, antimicrobial, anti-arthritic, in vivo study

1. INTRODUCTION

Inflammation is a critical area of biomedical science research, characterized by a network of molecular events and cellular activity. It is used to restore tissue, repair injuries, and regenerate new tissues.[1] The inflammatory cascade is preprogrammed and patterned, leading to tissue dysfunction and organ dysfunction. It is a popular therapeutic target, involving cellular functions like endocytosis, migration, division, and transformation. Previous studies have shown how the immune system causes inflammation, but further clinical literature is needed to understand specific processes.[2,3] Anti-inflammatory drugs, either Nonsteroidal or steroidal, are used for acute and chronic inflammations like rheumatoid arthritis and osteoarthritis.[4,5] Natural herbal sources are becoming more attractive for safer, more affordable, and effective medications. Traditional medicine, involving botanical extracts and active components, has shown various medicinal effects against various diseases and disorders.[6,7] Hydrogels have emerged as a versatile and promising platform for drug delivery and biomedical applications due to their high water content, biocompatibility, and tunable physical properties.[8,9] These polymeric networks can retain large amounts of water while maintaining their structure, making them ideal for topical and transdermal drug delivery.[10] The present study focuses on the formulation and evaluation of polyherbal hydrogels incorporating hydroalcoholic extracts from *Cordia obliqua*, *Tinospora cordifolia*, *Lantana camara*, *Sphaeranthus indicus*, and *Grewia abutilifolia*, which are traditionally used for their anti-inflammatory and antimicrobial properties.[11,12] Polyherbal formulations have been widely studied due to their synergistic effects, where multiple plant extracts work together to enhance therapeutic efficacy and reduce potential side effects.[13,14] In the context of hydrogel-based drug delivery, such formulations offer the advantage of controlled drug release, improved bioavailability, and enhanced patient compliance.[15,16] The incorporation of medicinal plant extracts into hydrogels presents a novel approach

for the treatment of inflammatory conditions and microbial infections.[17,18] The plants selected for this study have been well-documented for their pharmacological activities. *Cordia obliqua* is known for its wound-healing and anti-inflammatory effects,[19] while *Tinospora cordifolia* exhibits immunomodulatory and anti-oxidative properties.[20] *Lantana camara* has demonstrated significant antimicrobial and antifungal activities[21], *Sphaeranthus indicus* possesses potent anti-inflammatory and analgesic properties[22], and *Grewia abutilifolia* is traditionally used for its wound-healing and anti-bacterial effects.[23] The preparation of polyherbal hydrogels involves the use of Carbopol-940 as a gelling agent, along with ethanol, propylene glycol, methyl paraben, EDTA, propyl paraben, and triethanolamine for stabilization and preservation.[24,25] The formulation process ensures homogeneity and optimal physicochemical characteristics, such as pH, spreadability, and viscosity, to achieve efficient drug delivery.[26] To evaluate the efficacy of the formulated hydrogels, various in vitro and in vivo assays were conducted. The anti-inflammatory activity was assessed using protein denaturation and membrane stabilization assays.[27,28] Antimicrobial properties were determined through well diffusion assays against common bacterial strains, including *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus.*[29] In vivo studies, including acute dermal toxicity tests and carrageenan-induced rat paw edema models, were performed to establish safety and therapeutic efficacy.[30,31] This research aims to develop a scientifically validated, plant-based hydrogel formulation that the findings will contribute to the growing body of knowledge on polyherbal formulations and their applications in modern medicine, with a particular emphasis on their role in anti-inflammatory and antimicrobial therapies.

2. MATERIAL AND METHOD

2.1 Collection, authentication plant material and Sample preparation

All the required data and the process regarding the heading is already published in the first part of the paper in the "*REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504*
Vol 25, No.2 (2024)".

2.2 Preparation of hydrogel[32,33]

Six polyherbal hydrogel formulations were prepared using hydroalcoholic extracts from *Cordio oblique, Tinospora cardifolia, Lantana camara, Spharanthus indica,* and *Grewia abutifoloia.* 100 gm of gel was prepared by dissolving the extract in distilled water, adding Carbopol-940, ethanol, propylene glycol 400, methyl paraben, EDTA, propyl paraben, and tri-ethanolamine. The mixture was stirred at 500 rpm for 2 hours to achieve a homogeneous gel. Various formulation batches were prepared, with plant extracts excluded for the gel base. The gel formulations and base were stored at room temperature for 24 hours. The gel was then stirred with a propeller at 500 rpm for 2 hours to obtain a homogeneous gel.

2.3 Characterization of hydrogels[32,33]

2.3.1 Physical Appearance

2.3.1.1 Appearance and homogeneity

The prepared gels and control (base) were tested for physical appearance and homogeneity by visual observation

2.3.1.2 Homogeneity and appearance: The appearance of all hydrogel formulations was assessed through visual inspection. Homogeneity was evaluated by taking small amounts of the hydrogel formulations between the thumb and index finger, checking for the presence of any coarse particles. Additionally, a small amount of the hydrogel was applied to the back of the hand and rubbed in separately to observe its consistency.

2.3.2 pH:

The pH of all hydrogel formulations was measured utilizing a digital pH meter. One gram of each hydrogel was dissolved separately in distilled water and left to stand at room temperature for 2 hours to ensure complete dissolution and hydration of the hydrogel. The pH was then recorded in triplicate, and the average value was calculated.

2.3.3 Spreadability test

Spreadability was calculated by measuring the spreading area of 1.0 g hydrogel between two glass slides having diameter of both glass slides 19 cm x 19 cm. A standard weight of approximately 100.0 g was placed on the upper slide for 60 seconds to assess the Spreadability. The diameter of the dispersed gel was measured in cm and the result was calculated by using the following formula and their values were tabulated.

$$S=M.LTS=M.LT$$

Here, M = weight (g) placed on the upper glass slide, L = length in cm moved on the glass slide and T = time in seconds

2.3.4 Rheological study

Rheological study of all gels was performed by using Brookfield viscometer. The gel was placed into the Brookfield digital viscometer, which was then inserted into the viscometer's flow jacket. A sample adapter rotating at 20 rpm was used to measure the viscosity of the gel formulations. The temperature was maintained 24.8°C by rotating the water on the thermo stated water jacket. Sample was settled before 6 minutes to take readings. The viscosity of all hydrogels can be measured by increasing the value of the share rate.

2.3.5 Swelling index

One gram of gel was placed on porous aluminium foil and then immersed in a 50 ml beaker containing 10 ml of 0.1 N NaOH. Samples were taken from the beakers at various time intervals, allowed to dry for a while, and then reweighed.

Swelling Index (SW) % = [(Wt – Wo) / Wo] × 100.

Where, (SW) % = Equilibrium percent swelling,
Wo = Original weight of formulation at zero time after time t, Wt = Weight of swollen formulation

2.4 In *vitro* Anti-inflammatory activity

2.4.1 Protein denaturation assay

The study investigated the anti-inflammatory properties of crude plant extracts using a modified version of the BSA assay reported by **Williams *et al.*, 2008**[34]. The BSA solution was prepared in Tris Buffered Saline, adjusted to 6.4 with glacial acetic acid, and stock solutions of each plant extract were prepared in methanol. Different concentrations of stock solutions were added to test tubes containing a 0.4%, w/v BSA buffer solution. Both negative and positive controls were assayed in a similar manner. The solutions were heated in a water bath for 10 minutes and then cooled at room temperature. The turbidity of the solutions was measured at 660 nm using a Hach Spectrophotometer. The experiments were performed in duplicate, and mean absorbance values were recorded. The percentage inhibition of protein denaturation was calculated relative to the negative control using the equation:

% inhibition = 100 × (At / Ac - 1),

Where At represents the absorbance of the test sample and Ac represents the absorbance of the control. The concentration of the extract or drug required to achieve 50% inhibition (IC50) was determined by plotting the percentage inhibition relative to the control against the treatment concentration.

2.4.2 Membrane Stabilization Assay:

The study involved collecting 7 ml of blood from a healthy volunteer and preparing a stock erythrocyte suspension. The blood was centrifuged at 3000 rpm for 10 minutes, washed three times with an isotonic solution, and reconstituted to form a 40% (v/v) suspension using an isotonic buffer solution. The final prepared suspension was the stock erythrocyte suspension. The membrane-stabilizing activity of the extracts was evaluated using a hypotonic solution-induced haemolysis assay on human erythrocytes. 0.5 ml of stock erythrocyte suspension was combined with 5 ml of a hypotonic solution prepared in 10 mM sodium phosphate-buffered saline (pH 7.4) containing either the extracts (1.0 mg/ml) or acetylsalicylic acid (0.1 mg/ml). The absorbance of the supernatant was recorded at 540 nm using a UV spectrophotometer. The percentage inhibition of haemolysis, indicative of membrane stabilization, was calculated using the formula:

% inhibition = Absorbance of control - Absorbance of sample × 100.

The concentration of the methanolic extract, isolated compound, or drug (diclofenac sodium) required for 50% inhibition (IC50) was determined by plotting the percentage inhibition of haemolysis relative to the control against the treatment concentration. The IC50 value represents the potency of the substance in stabilizing the erythrocyte membrane.[35]

2.5 Antimicrobial Activity (Well Diffusion Assay)

2.5.1 Anti-bacterial Activity

The study involved preparing nutrient agar media by dissolving 28 g of media in distilled water and measuring its pH before sterilization. The media was then sterilized in an autoclave at 121°C under 15 lbs of pressure for 15 minutes. The nutrient media was then poured into plates and left to solidify. The bacterial suspension was standardized to 108 CFU/ml and transferred to a fresh, sterile agar media plate. Four wells, each 6 mm in diameter, were created in the inoculated agar and filled with different formulations (F1 to F5), with F6 serving as a control or placebo. The plates were left at room temperature for 30 minutes and incubated at 37°C for 18-24 hours. After incubation, the plates were examined for clear zones around the wells, indicating the antimicrobial activity of the formulations. The zone of inhibition (ZOI) was measured in millimeters and recorded.[36,37]

2.6 In vivo animal study

2.6.1 Animals

The animals, obtained from the in-house animal facility, were housed in standard, spacious, hygienic polypropylene cages and maintained at a temperature of 22 ± 2°C with a 12/12-hour light-dark cycle. They were provided with a commercially available normal pellet diet (NPD) from Keval Sales Corporation, Vadodara, and water was made available ad libitum throughout the study period.

2.6.2 Activity I: Acute dermal toxicity study (OECD 402)

2.6.2.1 Skin preparation for acute dermal toxicity study

The hairs on the dorsal skin surface (About 6 cm^2) of animals were carefully shaved 24 hrs using razor blade before application. According to OECD guidelines 402, approximately 10% of the body surface area should be left clear for the application of the test substance.

2.6.2.2 Experimental design for acute dermal toxicity

A study was conducted to assess dermal toxicity in rats following OECD guidelines. The positive control group received a dose of 2000 mg/kg of white soft paraffin 10% and a dose of Polyherbal hydrogel at the same dose. Rats were clinically checked on the first day and monitored daily for 14 days. Observations included behaviour patterns like salivation, tremors, convulsions, diarrhoea, lethargy, sleep, and coma. Physical appearance, injury, pain, signs of illness, skin, eyes, mucous membranes, respiratory rate, circulatory function, autonomic and central nervous system responses, and behaviour patterns were monitored daily.

2.6.3 Activity II: Complete Freund's adjuvant induced rat paw edema[38]

The study involved animals divided into five groups: normal, induced, standard, and test sample hydrogel (5%) and hydrogel (10%) treatment groups. The animals were given different hydrogel formulations, with Group I receiving a normal gel base, Group II receiving CFA 0.2 ml, Group III receiving standard drug diclofenac sodium gel, Group IV receiving formulation hydrogel (5%), and Group V receiving formulation hydrogel (10%). After arthritis induction, the animals were allowed to develop arthritis. After 14 days, polyherbal gel formulation and standard diclofenac sodium gel were applied topically until 21 days. During the experimental period, body weight and the rat paw volume of control and treatment groups were measured on Zero, 3rd, 7th, 14th and 21st day by using digital Vernier calliper. Anti-arthritic activity of polyherbal gel was evaluated on paw edema and arthritic score on day 0, 3, 7, 14 and day 21. On completion of the 21st day, blood samples were collected by retro-orbital puncture and the animals were sacrificed for paw histology.

2.6.3.1 Haematological Parameters

Blood was collected in EDTA-treated tubes for blood test. Blood parameters were quantified using an automatic haematological assay analyser.

2.6.3.2 Histopathology

The joint tissues were immersed in 10% formalin solution for histopathological examination. The tissues were processed by dehydrating them in varying concentrations of alcohol, clearing them in toluene, and then impregnating them with molten paraffin wax for the specified duration. The processed tissues were then embedded in fresh molten paraffin wax and allowed to solidify. Sections were at 3 µ and dried on a hot plate for 15 min and stained with hematoxylin and 1% aqueous eosin to demonstrate general tissue structure. Stained slides were dehydrated in various ascending grades of alcohol, cleared in xylene, and mounted in Canada balsam. Sections were viewed microscopically.[38,39]

2.6.4 Activity III: Carrageenan induced rat paw edema

After a seven-day acclimatization period, the animals were divided into five groups (n = 6/group): normal, induced, standard, and test groups receiving polyherbal gel (5% and 10%). Group I served as the normal untreated control, receiving only the normal gel base. Group II was treated with 0.1 ml of 2% (w/v) carrageenan solution and saline. Group III received a standard diclofenac sodium gel (locally purchased) applied topically 1 hour before carrageenan administration. Groups IV and V were treated with polyherbal gel at 5% and 10% concentrations, respectively, applied topically for seven days, with the final dose given 60 minutes prior to inflammation induction. Inflammation was induced by administering a subcutaneous injection of 0.1 ml of a 1% (w/v) carrageenan solution into the plantar region of the right hind paw of each animal. Paw volume was measured at 0, 1, 3, 5, and 7 hours post-injection using a Vernier calliper to assess edema formation.[40]

2.6.5 Statistical Analysis

Results are provided as Mean±SD (n=6). Results were analysed statistically using one-way analysis of variance (ANOVA) followed by Dunnett's t-test. $P < 0.05$ was considered as level of significance while comparison between groups.

3. RESULTS AND DISCUSSION

3.1 Formulation study

Table 1 Composition of the hydrogel formulation

S. No	Ingredients	PF1	PF2	PF3	PF4	PF5	PF6
1	**Ratio of the extracts *Cordio obliqua, Tinospora cardifolia, Lantana camara,Spharanthus indica andGrewia abutifoloia***	1:1:1:1:1	1:2:1:1:1	1:1:2:1:1	1:1:1:2:1	1:1:1:2:1	1:1:1:1:2
2	**Carbopol 940**	1gm	1 gm	1 gm	1 gm	1 gm	1 gm
3	**Propylene Glycol**	2ml	2ml	2ml	2ml	2ml	2ml

4	**Triethanolamine**	1.2	1.2	1.2	1.2	1.2	1.2
5	**Methyl paranben**	0.2	0.2	0.2	0.2	0.2	0.2
6	**Sodium benzoate**	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm	0.5gm
7	**Distilled water**	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml	Up to 100ml

Note: ratio: 1=100mg, 2=200mg of the plants extracts

Table 2 Physical characterization of formulations

S. No	Parameters	PF1	PF2	PF3	PF4	PF5	PF6
1.	Homogeneity	Slightly	Non homogeneous	Homogeneous	Slightly	Homogeneous	Slightly
2.	Grittiness	Non gritty	Non gritty	Non gritty	Non gritty	Non gritty	Non gritty
3.	Appearance	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent
4.	Color	Light brown	Dark brown	Slightly brownish	Pale yellow and greenish	Dark brown	Light brown
5.	Odor	Stringent	Stringent	Stringent	Stringent	Stringent	Stringent

It was observed that the freshly prepared formulations were light brown to dark brown in colour. All the formulation possesses stringent odour with translucent in appearance.

Table 3 pH of formulations

S. No	Parameters	PF1	PF2	PF3	PF4	PF5	PF6
1.	**pH:**	6.9±0.02	7.2±0.012	7.1±0.002	6.8±0.045	7.3±0.016	6.95±0.046

It was found to be in the range of 6.9 to 7.08, and the formulation code PF3 and PF5 shows the basic pH.

Table 4 Spread ability test of formulations

S. No	Parameters	PF1	PF2	PF3	PF4	PF5	PF6
1.	**Spreadability (mg/l)**	7.9±0.067	8.1±0.017	8.3±0.021	7.7±0.05	8.4±0.011	7.5±0.08

Spread ability of the base and formulations were studied and found to in the range of 7.5±0.08 to 8.4±0.011.All the formulations and base was found to possess good spread ability.

Table 5 Rheological study of formulations

S. No	Parameters	PF1	PF2	PF3	PF4	PF5	PF6
1.	**Viscosity (cps)**	4157±0.54	4145±0.87	4841±0.61	4152±0.85	4947±0.74	4745±0.78

Viscosity and Rheological properties of the formulations were found to be 4157±0.54 to 4947±0.74. The formulations PF3 and PF5 show the good viscosity rather than the other formulations.

Table 6 represent the swelling index of the transferosomal gel loaded with drug

S. No.	Parameters	PF1	PF2	PF3	PF4	PF5	PF6
1.	**Swelling Index (%)**	4.3	3.9	4.5	4.4	4.6	4.0

The swelling index of the formulation varied with the point value and among the formulation code PF2 and PF5 shows the better swelling index.

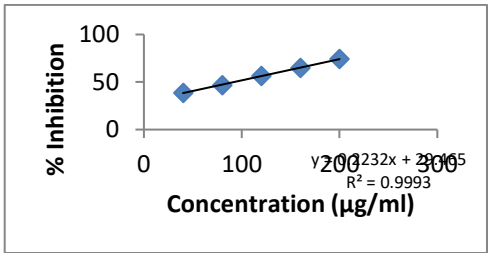
Note: After all the parameter of the characterization of polyherbal hydrogel it was clear that the formulation code PF3 and PF5 shows the better result.

3.2 Anti-inflammatory activity

3.2.1 Protein Denaturation Assay

Table 7 Protein denaturation activity of Diclofenac

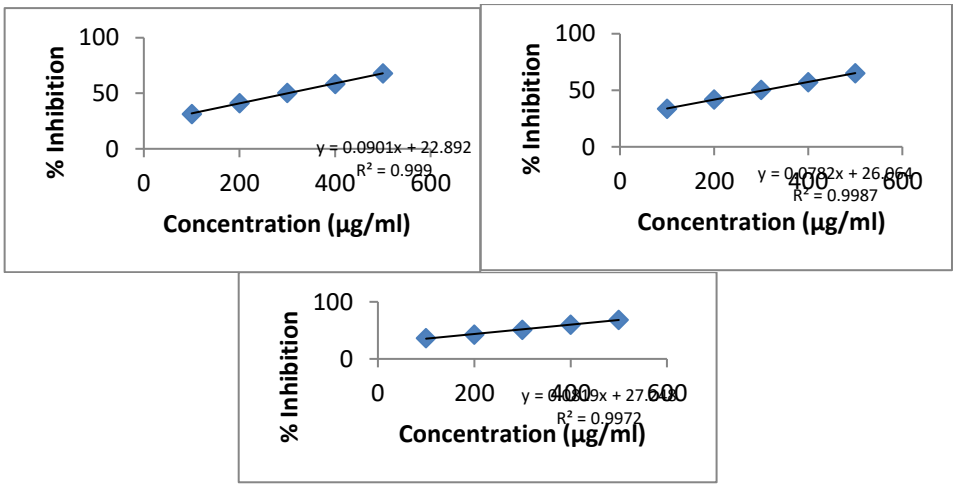
Concentration (µg/ml)	Absorbance	% Inhibition
40	0.481	38.726
80	0.417	46.878
120	0.342	56.433
160	0.276	64.840
200	0.201	74.394
Control	0.785	
IC50		92.107



Graph 1 Graph represents the Percentage Inhibition *Vs* Concentration of Diclofenac

Table 8 Protein denaturation activity of Formulation 1, 2 and 3

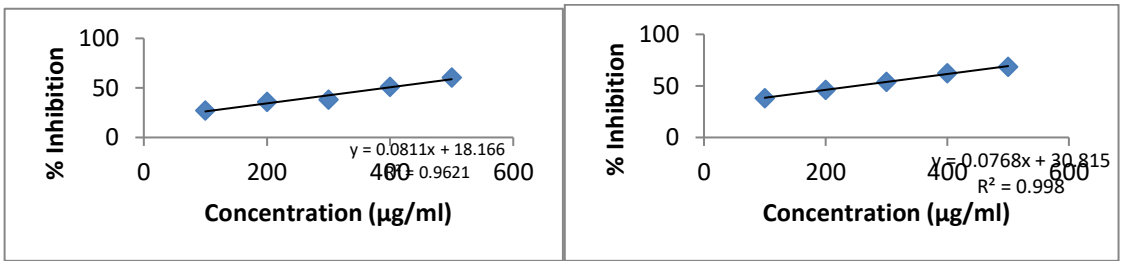
Concentration (µg/ml)	Formulation 1		Formulation 2		Formulation 3	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
100	0.538	31.464	0.522	33.503	0.5	36.305
200	0.461	41.273	0.456	41.910	0.449	42.802
300	0.389	50.445	0.391	50.191	0.382	51.337
400	0.326	58.471	0.338	56.942	0.314	60
500	0.252	67.898	0.274	65.095	0.246	68.662
IC50	**301.222**		**306.923**		**280.987**	
Control	**0.785**					

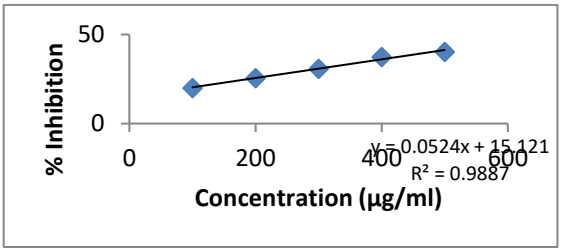


Graph 2 Graph represents the Percentage Inhibition *Vs* Concentration of formulation 1, 2 and 3

Table 9 Protein denaturation activity of Formulation 4, 5 and 6

Concentration (µg/ml)	Formulation 4		Formulation 5		Formulation 6 (Placebo)	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
100	0.571	27.261	0.485	38.216	0.628	20
200	0.503	35.923	0.423	46.114	0.584	25.605
300	0.4865	38.025	0.36	54.140	0.543	30.828
400	0.384	51.082	0.296	62.292	0.491	37.452
500	0.312	60.254	0.247	68.535	0.469	40.254
IC50	**393.086**		**252.500**		**670.769**	
Control	**0.785**					



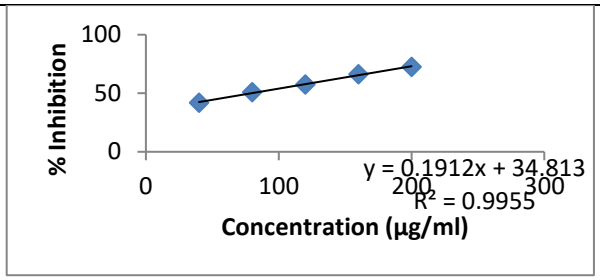


Graph 3 Graph represents the Percentage Inhibition *Vs* Concentration of formulation 4, 5 and 6

3.2.2 Membrane stabilization Assay

Table 10 Membrane stabilization assay of Diclofenac

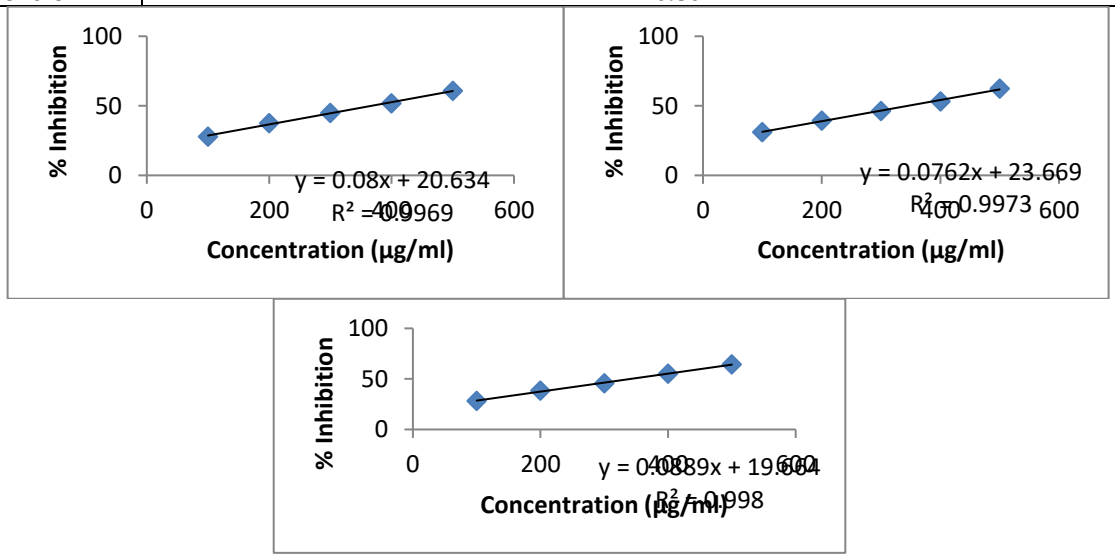
Concentration (µg/ml)	Absorbance	% Inhibition
40	0.468	41.791
80	0.394	50.995
120	0.343	57.338
160	0.271	66.293
200	0.222	72.388
Control	**0.804**	
IC50		**76.14**



Graph 4 Graph represents the Percentage Inhibition *Vs* Concentration of Diclofenac

Table 11 Membrane stabilization assay of Formulation 1, 2 and 3

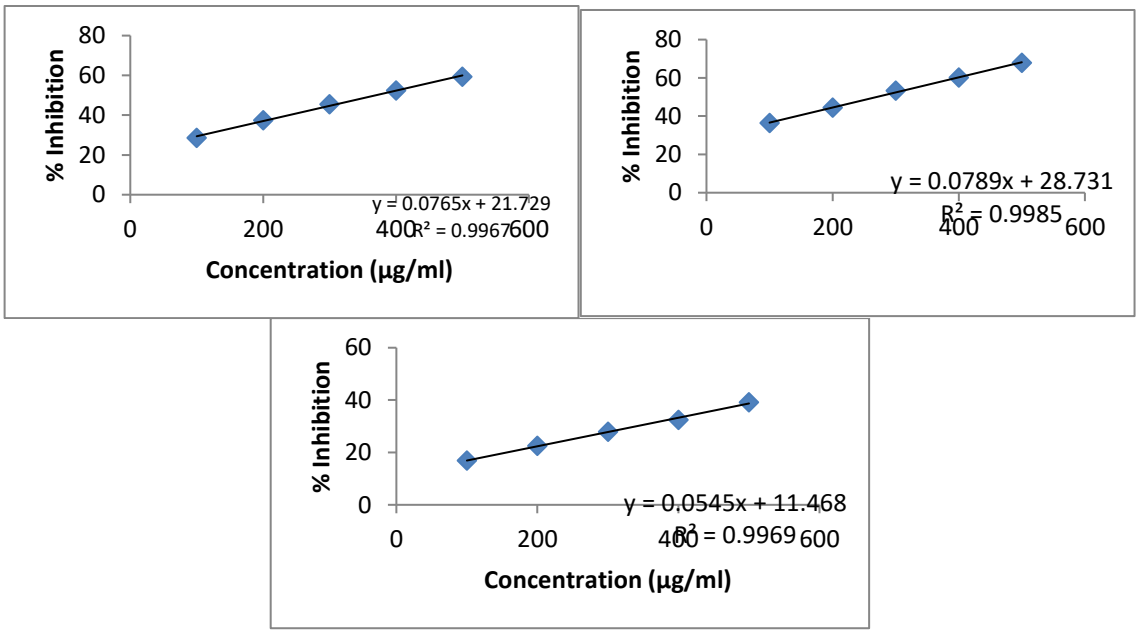
Concentration (µg/ml)	Formulation 1		Formulation 2		Formulation 3	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
100	0.579	27.985	0.553	31.218	0.576	28.358
200	0.502	37.562	0.487	39.427	0.496	38.308
300	0.443	44.900	0.431	46.393	0.438	45.522
400	0.387	51.865	0.376	53.233	0.361	55.099
500	0.315	60.820	0.302	62.437	0.286	64.427
IC50	**367.125**		**346.578**		**344.772**	
Control	**0.804**					



Graph 5 Graph represents the Percentage Inhibition *Vs* Concentration of formulation 1, 2 and 3

Table 12 Membrane stabilization assay of Formulation 4, 5 and 6

Concentration (µg/ml)	Formulation 4		Formulation 5		Formulation 6 (Placebo)	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
100	0.574	28.606	0.512	36.318	0.668	16.915
200	0.503	37.437	0.447	44.402	0.623	22.512
300	0.438	45.522	0.376	53.233	0.579	27.985
400	0.382	52.487	0.321	60.074	0.543	32.462
500	0.327	59.328	0.258	67.910	0.489	39.179
IC50	**372.105**		**272.692**		**713.703**	
Control	**0.804**					



Graph 6 Graph represents the Percentage Inhibition *Vs* Concentration of formulation 4, 5 and 6

3.3 Antibacterial activity of Formulations

Table 13 Anti-bacterial activity of formulations against *P. aeroginosa*

Formulations	Zone of Inhibition in mm			
	Plate 1	Plate 1	Plate 2	Mean±SD
F1	0	0	0	0±0
F2	0	0	0	0±0
F3	7	8	7	7.333±0.577
F4	0	0	0	0±0
F5	7	7	7	7±0
Control	0	0	0	0±0

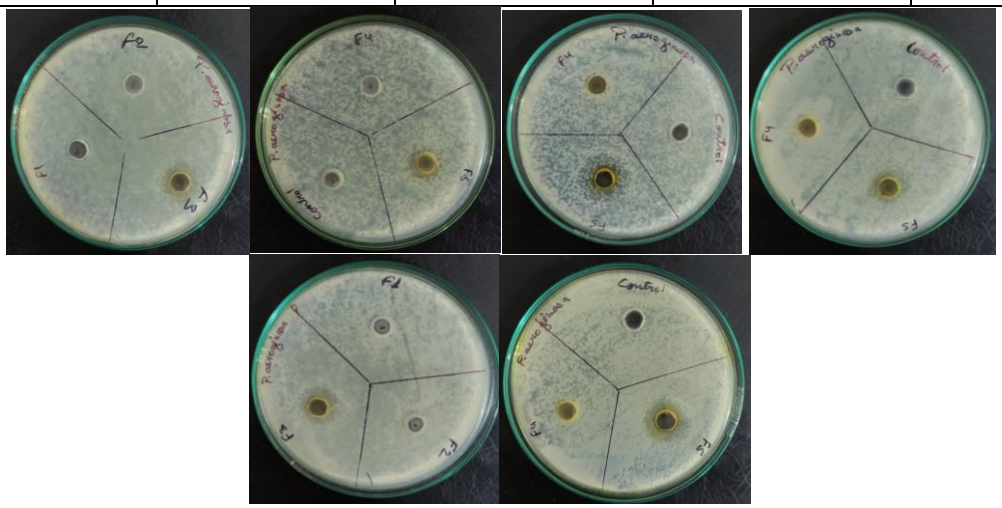


Table 14 Anti-bacterial activity of formulations against *E. coli*

Formulations	Zone of Inhibition in mm			
	Plate 1	Plate 1	Plate 2	Mean±SD
F1	0	0	0	0±0
F2	0	0	0	0±0
F3	7	8	7	7.333±0.577
F4	0	0	0	0±0
F5	12	11	13	12±1
Control	0	0	0	0±0

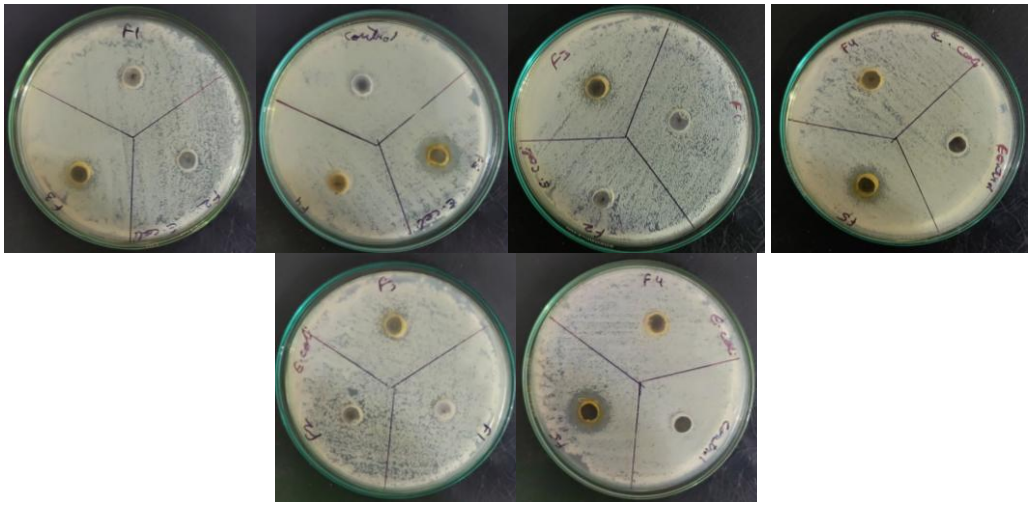
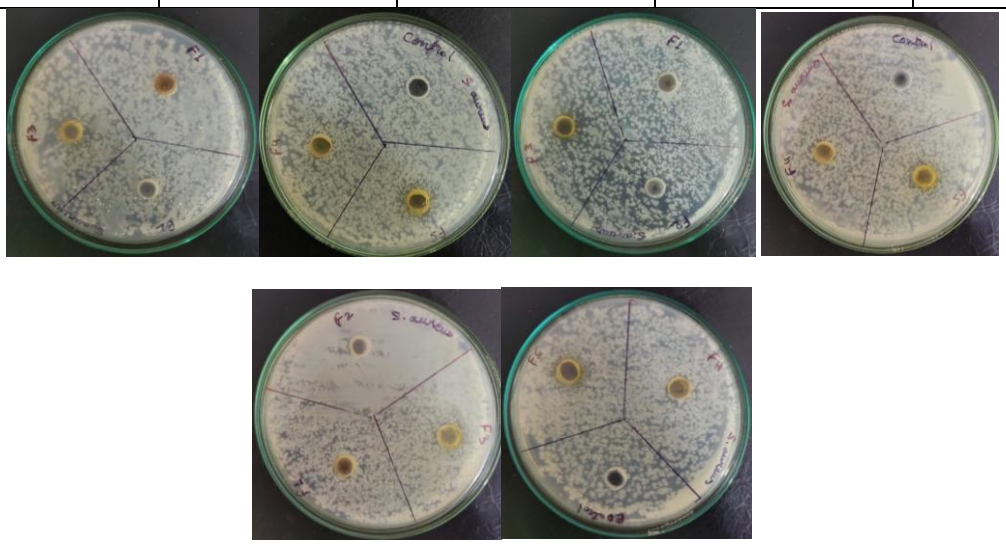


Table 15 Anti-bacterial activity of formulations against *S.aureus*

Formulations	Zone of Inhibition in mm			
	Plate 1	Plate 1	Plate 2	Mean±SD
F1	0	0	0	0±0
F2	0	0	0	0±0
F3	7	6	6	6.333±0.577
F4	0	0	0	0±0
F5	7	6	7	6.666±0
Control	0	0	0	0±0



3.4 Acute dermal toxicity study

The study found no significant changes in behavior, skin, fur, eyes, or behavior in rats treated with polyherbal gel. No mortality was observed after 14 days, and the gel should be labeled as unclassified nontoxic according to the OECD-hazard.

3.5 *In-vivo* anti-arthritic activity

3.5.1 Joint measurement

In Freund's adjuvant induced arthritis, an increase in size in joint section was observed in arthritic rats as compared to control rats throughout the experiment. The results revealed that topical application of polyherbal gel 10% orally for 21 days and polyherbal gel 5% reduces the complication associated with arthritis by inhibiting the edema formation. Weekly assessment of knee joint swelling was done using digital calliper.

Table 16 Knee Joint measurement (mm) of rats at 0, 3, 7, 14 and 21 days

S. No.	Group	Knee Joint (mm)				
		0 day	3 day	7 day	14 day	21 day
I	Normal Control	6.08±0.555	6.09±0.518	6.06±0.565	6.09±0.558	6.08±0.568
II	CFA (0.2 ml)	6.20±0.384	7.51±0.469	7.88±0.604	9.55±0.522	9.94±0.559
III	Standard Diclofenac sodium	6.24±0.513	7.19±0.513	7.56±0.559	6.55±0.828*	6.14±0.682*
IV	Polyherbal gel (5%)	6.20±0.411	7.51±0.439	7.95±0.408	7.79±0.688	7.00±0.626*
V	Polyherbal gel (10%)	6.16±0.525	6.76±0.552	7.12±0.457	6.93±0.501	6.47±0.456*

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Dunnett's test, **P<0.050 compared to the CFA induced

3.5.2 Effects of polyherbal gel on the levels of RBCs, WBCs, Hb and ESR in arthritic rats

Levels of red blood cells (RBC), white blood cells (WBC), and haemoglobin (Hb) in CFA-induced arthritic rats were estimated

Table 17 Alteration of hematological parameters in Anti-arthritic study

Group No.	Group	Parameter			
		Hb	RBC	WBCS	ESR
1.	Normal Control	16.60±0.951	12.27±1.919	6.17±0.755	5.15±0.787
2.	CFA (0.2 ml)	7.97±0.852	4.73±0.597	11.47±0.607	10.74±1.677
3.	Standard Diclofenac sodium	13.37±1.363*	9.90±0.900	6.77±0.717**	4.92±0.639
4.	Polyherbal gel (5%)	13.20±0.796*	11.72±1.527*	6.52±0.373**	5.20±0.878
5.	Polyherbal gel (10%)	16.32±0.929**	12.39±1.678*	5.92±0.662**	5.10±0.752

Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Dunnett's test, **P<0.050 compared to the CFA induced

3.5.3 *In-vivo* anti-arthritic activity (Paw edema)

An increase in paw volume was observed in Carrageenan-induced arthritic rats compared to control rats throughout the experiment. The results revealed that continuous topical application of polyherbal gel 10 % and 5 %reduces the complication associated with arthritis by inhibiting the edema formation as shown in Table 8.

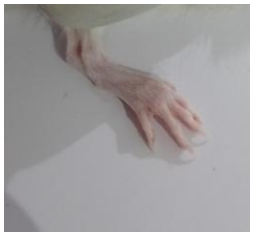
Table 18 Paw edema (mm) of rats at 0, 1, 3, 5 and 7 hours

S.No.	Group	Paw edema (mm)				
		0hr	1hr	3hr	5hr	7hr
I	Normal Control	4.08±0.060	4.09±0.067	4.08±0.051	4.09±0.037	4.08±0.022
II	Carrageenan (0.1 ml)	4.09±0.019	4.81±0.232	5.34±0.280	5.81±0.367	6.40±0.185
III	Standard Diclofenac sodium	4.03±0.060	4.70±0.133	5.03±0.197	4.41±0.161*	4.13±0.075**
IV	Polyherbal gel (5%)	4.07±0.048	4.72±0.149	5.39±0.400	4.99±0.124	4.37±0.311**
V	Polyherbal gel (10%)	4.05±0.045	4.68±0.118	5.30±0.372	4.80±0.350	4.29±0.329**

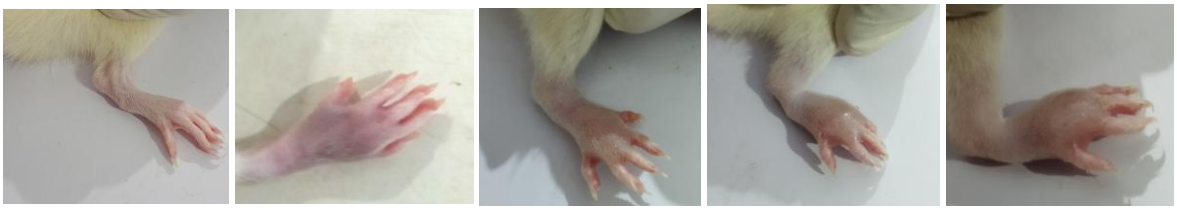
Values are expressed as MEAN±SD at n=6, One-way ANOVA followed by Dunnett's test, **P<0.050 compared to the CFA induced

3.6 Images

Normal Control



CFA induced

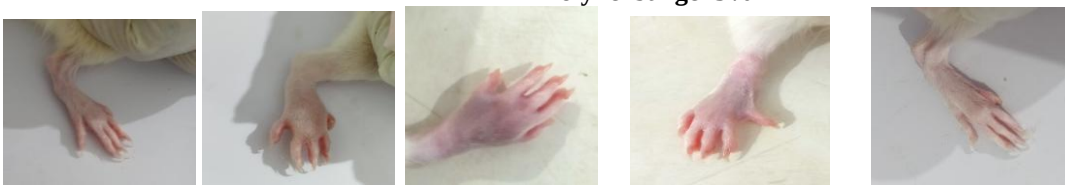


0 hr, 1 hr, 3 hr, 5 hr, 7 hr

Standard Indomethacin

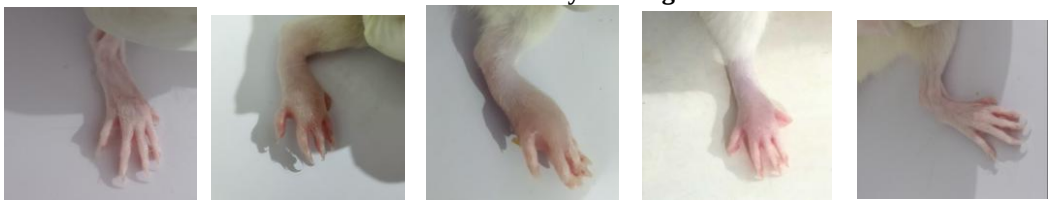
0 hr, 1 hr, 3 hr, 5 hr, 7 hr

Polyherbal gel 5%



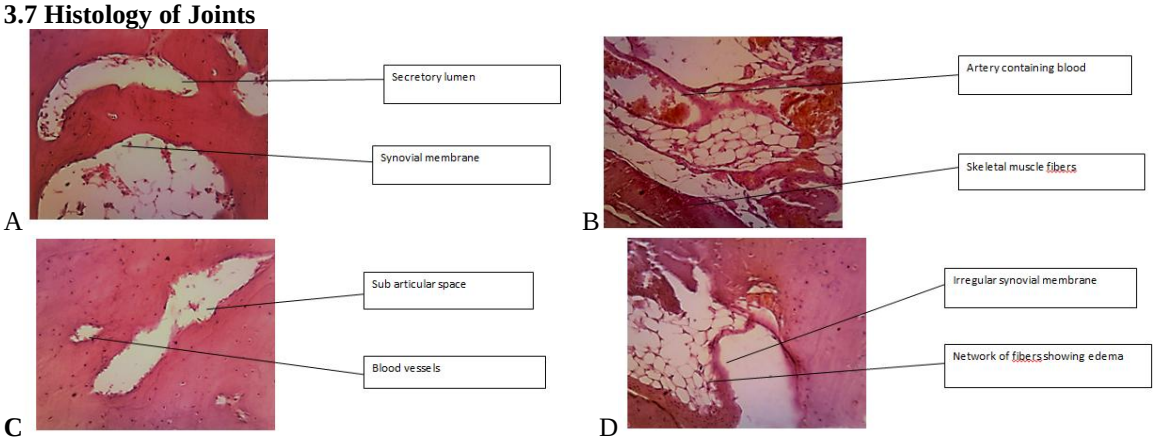
0 hr, 1 hr, 3 hr, 5 hr, 7 hr

Polyherbal gel 10 %

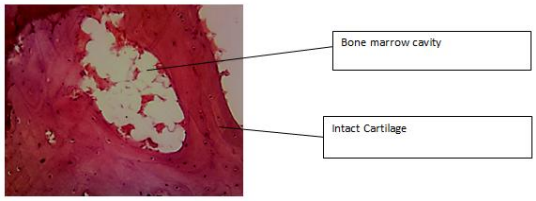


0 hr, 1 hr, 3 hr, 5 hr, 7 hr

3.7 Histology of Joints



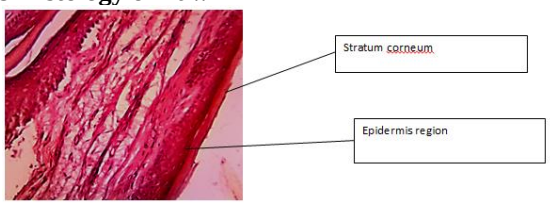
A B C D



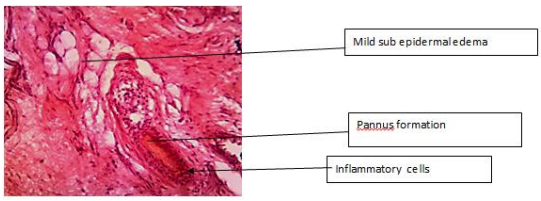
E

- **A. Normal control** showed smooth articulation of the joint cartilage surface, regular joint space with the normal connective tissue of the synovial membrane
- **B. CFA induced** Distortion is observed in Freuds adjuvant treated animals and edema, inflammatory cells
- **C. Standard treated showing** the chondroblasts in the outer surface and inner surface of the articular cartilage
- **D. 5% Polyherbal gel showing** inflammatory cells in synovial cavity and less soft tissue swells.
- **E. 10 % Polyherbal gel** showed less inflammatory cell infiltration, minimal synovial hyperplasia

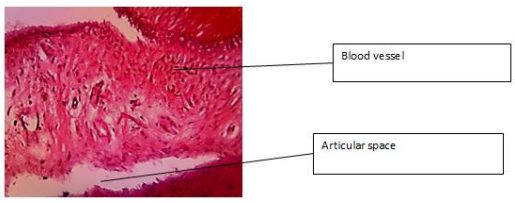
3.8 Histology of Paw



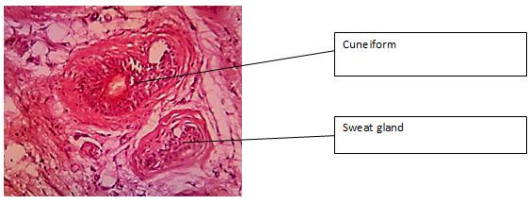
A



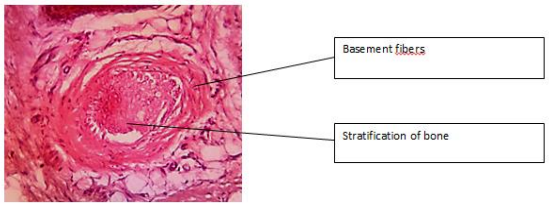
B



C



D



E

- **A. Normal control:** showing stratum corneum, normal epidermis region
- **B. Negative control:** Carrageenan induced showing Pannus formation, it is filled with inflammatory exudate composed of cell debris
- **C. Standard treated group** showing clear articular spaces, cortical bone and intact cartilage
- **D. Polyherbal gel 5% treated rats showing normal bone marrow, cuneiform and swaet glands**
- **E. Polyherbal gel 10 % treated rats showing** loose connective tissue, stratified bone and collagen fibers

4. DISCUSSION

The study successfully developed a polyherbal hydrogel incorporating hydro alcoholic extracts from medicinal plants to evaluate its anti-inflammatory, antimicrobial, and anti-arthritic properties. The formulated hydrogel was characterized for its physicochemical properties, including pH, Spreadability, viscosity, and swelling index, with formulations PF3 and PF5 exhibiting superior characteristics. In vitro assessments demonstrated significant anti-inflammatory activity, with PF5 showing the highest inhibition of protein denaturation and membrane stabilization. The hydrogel also exhibited antibacterial activity, particularly against *E. coli* and *S. aureus*. In vivo toxicity studies confirmed the safety of the formulations, with no observed toxic effects. Furthermore, in vivo anti-arthritic evaluation revealed a reduction in paw edema and joint inflammation in treated groups, with PF5 demonstrating superior efficacy compared to the standard diclofenac sodium gel. Histopathological analysis supported these findings by showing reduced inflammation and improved joint tissue conditions in treated subjects. The results suggest that PF5 is the most promising formulation, highlighting its potential as a safe and effective topical therapeutic agent for inflammatory disorders. Further pharmacological and clinical studies are warranted to establish its therapeutic applicability.

5. CONCLUSION

The development of the polyherbal hydrogel demonstrated significant anti-inflammatory, antimicrobial, and anti-arthritic potential, highlighting its efficacy as a promising topical therapeutic agent. Among the tested formulations, PF5 exhibited

superior performance in terms of protein denaturation inhibition, membrane stabilization, and antibacterial activity, particularly against *E. coli* and *S. aureus*. The in vivo studies further confirmed the hydrogel's safety and effectiveness in reducing paw edema and joint inflammation, with PF5 showing comparable or better results than standard diclofenac sodium gel. These findings support the potential of polyherbal hydrogels in managing inflammatory disorders and warrant further clinical investigations for therapeutic applications.

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