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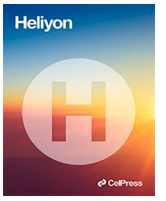

Research article

Preclinical approaches to evaluate *Uncaria tomentosa* bark extract loaded FDOFs using osteoarthritis models

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ABSTRACT

Osteoarthritis (OA) is one of the biggest global health issues, affecting two thirds of the population and cannot be fully treated to return normal function or relieve joint discomfort. Oral fast-dissolving films offer a high medication loading capacity, targeted distribution, and increasing bioavailability. The current research explores the in vitro and in vivo efficacy of oral fast-dissolving film formulations containing *Uncaria tomentosa* bark extract. A three-dimensional osteoarthritis (OA) model was created for in vitro assessment using first passage chrondrocytes cultured in a 1:1:3 ratio on trypsin-EDTA medium. C20A4 chondrocytes were cultured on agarose gel at 25± 5 °C in a phosphate buffer solution to create the OA agarose model. One milliliter of RPMI-1640 (10 % FBS) was used for chrondrocyte cultivation. On the third day of incubation, a 20 % (IL-1β) solution was applied, and the media was periodically changed. On the fifth day of incubation, the treated cell line received 5 μL of 0.5 % MTT reagent, and absorbance was examined at 570 nm. The effects of FDOFs on the cell lines were examined for 7, 13, 27, and 35 days (IL-1β, F5, F13 treated IL-1β injected types and Control). As a control, chondrocytes in agarose constructs were solely grown in RPMI-FBS medium without IL-1β. The arthritic effect of improved FDOFs, i.e., F5, was investigated using the GAG, HYP, and DNA quantitation assays in conjunction with a DNA content assay. Monoiodoacetate (MIA) produced arthritis models are well-established for understanding weight bearing and reaction to tactile stimuli in invivo research. Seven-week-old male wistar rats were used in the *invivo* technique, with celecoxib serving as the positive control and MIA as the negative control. Estimates of osteoarthritic potential were made based on the evaluation of knee thickness and discomfort. The study's findings demonstrated the effectiveness of the F5 formulation on OA models, which need for a clinical evaluation in human beings.

1. Introduction

Osteoarthritis (OA) is a chronic, degenerative disease of the joints and surrounding tissue that affects millions of people. It is considered a major and demanding concern [1]. In actuality, there is now no effective medical treatment for this degenerative joint illness. However, There are numerous documented long-term therapies that can ease discomfort or lessen its frequency including the intraarticular injection of hyaluronan (HA). The illness is described as due to a gradual loss of joint cartilage that causes deformity, dysfunction, and pain which were not defeated by oral medication therapies that were standard. As a result, there is an increase in

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alternate therapies to deal with this problem by changing the oral medications that have a delayed release polymers that have a long half-life and can release and hold onto drugs [2,3]. Furthermore, the use of *Uncaria tomentosa* (cats claw) extract in the treatment of inflammatory bowel disease and arthritis is well established [4,36]. It has been observed that the plant is rich in phytochemical like alkaloids, polyphenols, flavanoids, sterols, and terpenoids [6].The plant contains pseudoindoline alkaloids, such as mitraphylline and pteropodine, which have anti-inflammatory and apoptotic properties and are important in the treatment of rheumatoid arthritis. The oral fast dissolving films are more readily available than traditional tablets and offer better patient compliance is therefore thought to be the more effective method of resolving issues with osteoarthritis, particularly for the treatment of pain [5]. Since the API can be soluble or insoluble, this innovative method can be extended to create even the herbal supplements. The oral bioavailability can be improved by utilizing appropriate film-forming polymers, plasticizers, and disintegrants. New medication administration methods that are site-specific, have better patient compliance, and can be a practical application in the elders [7]. The main impediment and driving force behind the treatment plan's orientation toward the replacement procedure is pain [8]. Two forms of pain are distinguished. a dull aching that eventually turns into a shorter, throbbing pain that is frequent happening in three phases: progressive, mid, and early. The primary components of the treatment protocol are Joint replacement therapy, NSAIDS, steroidal injections, and lifestyle modifications at chronic stage [8,9]. A better course of treatment is having a thorough understanding of chronic pain management [10]. Consequently, the assessment procedures for osteoarthritis comprise identifying a well-defined preclinical model of the condition, such as invivo protocols (which entail pathogenic inflammatory mediators like TNF-α, IL-6, and IL-1) and *invitro* protocols (which involve technique for managing pain [11,12]. The current research used the Box Behnken design in formulation and characterisation of fast oral dissolving films of Uncariatomentosa bark extract [13,26]. In addition, in vitro *and* in vivoevaluation of fast dissolving oral films of *Uncaria tomentosa* bark extract was performed in order to demonstrate the product's potential as a viable commercial product and as an option to the currently available osteoarthritis therapy solution.

2. Materials and methods

All the equipments, chemicals, and materials utilized for the research were of high purity with laboratory analytical grade. The equipment was sanitized before being used. ANOVA [14,15] was used for the statistical analysis.

2.1. Developing FDOFs through QbD design and characterisation

Fourteen *Uncaria tomentosa* bark extract fast dissolving oral films were formulated and optimized by applying QbD Box Behnken design. Films are prepared by solvent casting method. Folding endurance and disintegration time were represented as dependent variables with various natural film forming agents, synthetic polymers, super disintegrants, plasticizers as independent variables. Formulation characteristics like physical appearance, mechanical behaviour of films and percentage drug release were evaluated. Utilizing the design expert program, 3D contour plots and response curves were examined, demonstrating that the ideal oral dissolving films of extract with Pullalan gum, Hydroxy Propyl Methyl Cellulose (polymers), Propylene glycol, PEG 400 (co-solvents) and Super Disintegrant croscarmellose sodium are consistent and stable in its formulation characteristics. The drug release rates demonstrate that the F5 and F13 formulations had adequate mechanical qualities and follows first order kinetics, resulting in 99.90 % drug release in 30 min (Tables 1–3).

3. Evaluation of in-vitro osteoarthritis activity

3.1. Maintenance and cultivating of C20A4 chondrocytes

The National Centre for Cell Sciences in Pune, India provided the human chondrocyte cell lines C20A4. In RPMI-1640 (10 % fetal bovine serum (FBS) and 10 U antibiotic/ml), chondrocytes were cultivated. (Aldrich Sigma) in an incubator (5215, Shel Lab, USA) with 5 % CO_2 at 37 °C. The medium of growth was replaced every three daysand the chondrocytes were passage with trypsin-EDTA solution (0.05 %) in a 1:3 ratio [16]. Cell culture experiments were conducted using first passage chondrocytes.

3.2. Three-dimensional in-vitro osteoarthritis model

To conduct in-vitro experiments, a 3- dimensional (3D) OA agarose model (Fig. 1a and b) was developed using C20A4chondrocytes of Osteoarthitis. C20A4chondrocytes [17] were embedded in agarose (2 % low-melting agarose-gelling temperature 25± 5 °C) (Sigma, USA) prepared in phosphate buffer solution. Next, to achieve a final chondrocyte concentration of 10^6 cells per mL in each well of a 24-well plate, equal amounts of chondrocyte suspension in double strength RPMI1 1640 (20 % FBS) were combined with agarose. Subsequently, 1 mL (10 percent FBS) of RPMI-1640 was added to every well. Three days a week, Medium was updated. During the third day of the in-vitro culture, 20 ng/mL of interleukin-1β (IL-1β) was introduced into the medium. Every time the medium was changed, the same quantity of IL-1β was added.

3.3. Tests for cell viability

In contact with optimized formulations F5 and F13 at several doses (Fig. 2) ranging from 0 to 500 μg/mL, the cell viability [18–20] of C20A4 chondrocytes as well as the IL-1β-induced OA model of C20A4 were evaluated (0, 10, 50, 100, 200, 350 and 500 μg/mL. The

Table 1
Composition of Fast dissolving oral films (FODFs).

S. No	Formulation entry	Category	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14
1	Cats claw extract	API	100	100	100	100	100	100	100	100	100	100	100	100	100	100
2	Pullulan gum	Natural thickening agent	100	–	–	–	100	–	–	–	100	–	–	–	50	–
3	Maltodextrin	Thickening agent	–	100	–	–	–	100	–	–	–	100	–	–	–	50
4	HPMC	Polymer	–	–	100	–	–	–	100	–	–	–	100	–	50	–
5	PVA	Polymer	–	–	–	100	–	–	–	100	–	–	–	100	–	50
6	Propylene glycol*	Penetration enhancer	10	10	10	10	–	–	–	–	10	–	10	–	10	10
7	PEG 400*	Penetration enhancer	–	–	–	–	10	10	10	10	–	10	–	10	–	–
8	Cross Povidone	Disintegrant	10	10	10	10	–	–	–	–	–	–	–	–	–	–
9	Croscarmellose sodium	Disintegrant	–	–	–	–	10	10	10	10	–	–	–	–	10	10
10	Pregelatinized starch	Disintegrant	–	–	–	–	–	–	–	–	10	10	10	10	–	–
11	Citric acid	Preservative	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
12	Polysorbate 80*	Surfactant, emulsifier	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
13	Bronopol	Antimicrobial	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
14	Sucralose	Artificial sweetener	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
15	Distilled water*		Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Quantities were mentioned in mg/film, *ml/film.

Table 2
Characteristic formulation features of *Uncaria tomentosa* Fast Dissolving Oral Films.

F. code	Film forming capacity	Appearance of films	Tackiness	thickness (mm)	Surface pH	% moisture loss	% moisture gain
F1	Good	Transparent	Non-tacky	0.06 ± 0.013	6.71 ± 0.35	15.6 ± 1.31	12.9 ± 157
F2	Very good	Transparent	Non-tacky	0.07 ± 0.021	6.52 ± 0.23	9.3 ± 1.12	10.7 ± 0.83
F3	Very good	Transparent	Non-tacky	0.08 ± 0.025	6.35 ± 0.18	13.9 ± 2.44	17.2 ± 0.99
F4	Good	Transparent	Non-tacky	0.09 ± 0.019	7.01 ± 0.21	11.2 ± 2.86	11.5 ± 0.83
F5	Very good	Transparent	Non-tacky	0.06 ± 0.023	7.03 ± 0.31	6.5 ± 0.75	6.2 ± 0.54
F6	Very good	Transparent	Slightly tacky	0.08 ± 0.016	6.84 ± 0.17	19.3 ± 1.56	12.5 ± 1.13
F7	Good	Transparent	Non-tacky	0.09 ± 0.019	7.69 ± 0.42	14.9 ± 1.22	11.9 ± 1.05
F8	Good	Transparent	Non-tacky	0.08 ± 0.021	6.29 ± 0.25	9.7 ± 0.54	7.3 ± 0.85
F9	Good	Transparent	Non-tacky	0.09 ± 0.025	7.62 ± 0.29	13.9 ± 1.29	15.2 ± 1.36
F10	Good	Transparent	Non-tacky	0.08 ± 0.016	7.42 ± 0.24	17.5 ± 158	13.7 ± 1.59
F11	Very good	Transparent	Slightly tacky	0.07 ± 0.023	7.48 ± 0.39	18.4 ± 1.66	12.4 ± 1.09
F12	Good	Transparent	Non-tacky	0.09 ± 0.026	6.85 ± 0.32	15.4 ± 1.27	13.8 ± 0.95
F13	Very good	Transparent	Non-tacky	0.07 ± 0.018	7.19 ± 0.39	5.3 ± 0.47	6.9 ± 0.58
F14	Average	Transparent	Slightly tacky	0.09 ± 0.024	7.75 ± 0.21	26.1 ± 1.89	17.2 ± 1.32

The data is represented as Mean ± S.D (n = 3).

Table 3
3^3 factorial design Uncaria tomentosa Fast Dissolving Oral Films.

F. Code	Independent variable			Dependant variable	
	X1 (Polymer)	*X2 (Plasticizer)*	*X3 (Superdisintigrant)*	*Y1 (Folding endurance)*	*Y2 (Disintegration time in sec)*
F1	Pullulan gum	Propylene glycol	Cross Povidone	170.5 ± 11.6	41.2 ± 1.26
F2	Maltodextrin	Propylene glycol	Cross Povidone	205.6 ± 13.6	52.3 ± 2.31
F3	HPMC	Propylene glycol	Cross Povidone	190.4 ± 15.5	48.1 ± 2.54
F4	PVA	Propylene glycol	Cross Povidone	165.5 ± 12.4	56.5 ± 2.95
F5	Pullulan gum	PEG 400	Croscarmellose sodium	215.2 ± 13.9	35.9 ± 0.58
F6	Maltodextrin	PEG 400	Croscarmellose sodium	210.3 ± 11.2	59.7 ± 3.65
F7	HPMC	PEG 400	Croscarmellose sodium	220.5 ± 13.4	47.8 ± 2.28
F8	PVA	PEG 400	Croscarmellose sodium	200.7 ± 15.1	63.8 ± 3.84
F9	Pullulan gum	Propylene glycol	Pregelatinized starch	185.6 ± 14.3	66.1 ± 3.24
F10	Maltodextrin	PEG 400	Pregelatinized starch	210.1 ± 14.7	54.1 ± 1.92
F11	HPMC	Propylene glycol	Pregelatinized starch	240.3 ± 11.4	48.3 ± 3.25
F12	PVA	PEG 400	Pregelatinized starch	210.7 ± 15.8	58.1 ± 2.82
F13	Pullulan gum + HPMC (50:50)	Propylene glycol	Croscarmellose sodium	240.5 ± 18.3	34.1 ± 2.31
F14	Maltodextrin + PVA (50:50)	Propylene glycol	Croscarmellose sodium	185.3 ± 14.7	75.3 ± 4.51

The data is represented as Mean ± S.D (n = 3).

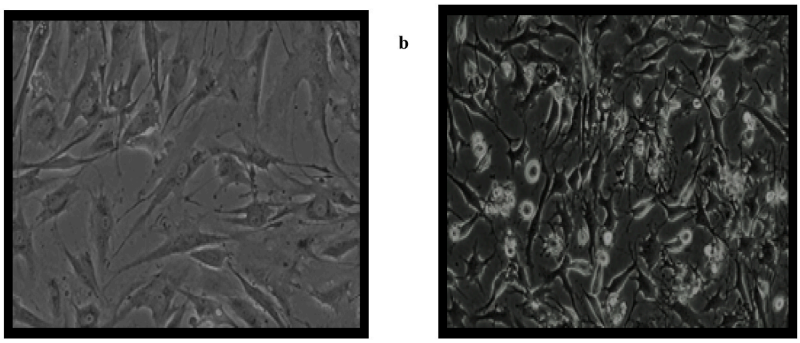

Fig. 1. Culture of Human chondrocyte cell lines C20A4 in RPMI media without IL-1β treatment (a); With IL-1β treatment (b).

C20A4, and C20A4/IL-1β cells were plated The C20A4, and C20A4/IL-1β cells were plated in 96-well plates at a density of 20,000 cells/well. After generating OA, C20A4/IL-1β cells were treated for five days with IL-1β. After the culture was incubated for 24 h, F5 and F13 were suspended in PBS and kept at 37 °C for another 24 h. Approximately 5 μL of 0.5 % 3-(4,5-dimethylthiazol-2-yl)- 2, 5-diphenyltetrazolium bromide (MTT) solution was added to each well, and the plates were kept aside for 3 h. After 45 min of incubation with 1 μL of DMSO in each well, the absorbance was measured at 570 nm on a Sunrise Tecan microplate reader.

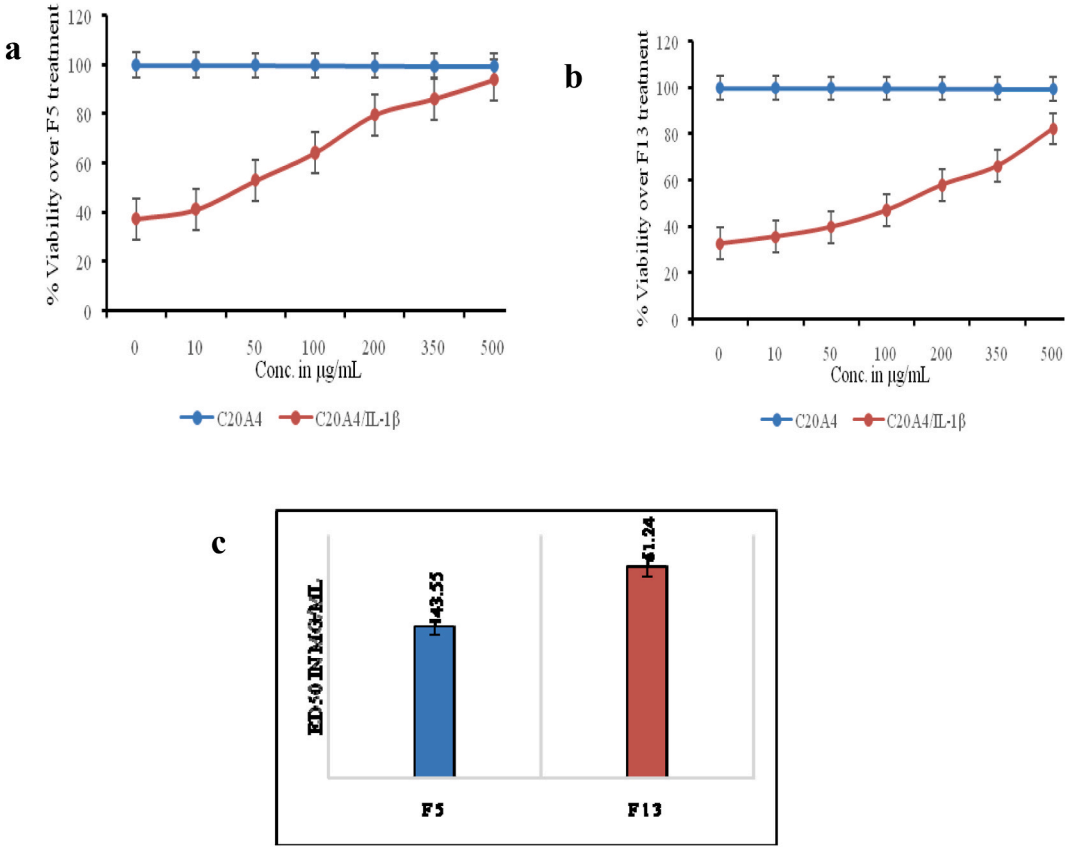

Fig. 2. Assay of Cell viability of chrondrocytes with a) F5 formulation treatment b) F13 formulation treatment c)Effective growth.

3.4. Analysis procedure

Efficacy of the release systems and In vitro establishment of OA model [21–23] were assessed after 7, 15, and 23 days of incubation. Cell lines were split into four groups to determine the impact of FDOFs: IL-1β, F5, and F13 treated IL-1β injected cell lines and control. As a control, chondrocytes in agarose constructions that were solely grown in RPMI-FBS medium without IL-1b were used. The media for the analysis of GAG, hydroxyproline (HYP), and MMP-13 were gathered and kept at −80 °C. In order to prepare agarose constructs containing chondrocytes for GAG, HYP, and DNA quantification assays, they were papain digested. The results of GAG and collagen levels were normalized using the amounts of chondrocyte DNA in agarose.

3.5. Assay for DNA content

The amount of DNA in the papain-digested samples was measured using a fluorometer (Modulus, USA) and Hoechst 33258 dye (Invitrogen, Germany). As a reference, calf thymus DNA was utilized. The measurements were made in compliance with the guidelines provided by the instrument and DNA concentrations were expressed in units of (µg) [20].

3.6. The GAG test, or glycosaminoglycan assay procedure

The 1,9dimethylmethylene blue (DMMB) test was used to measure the total quantities of sulphated glycosaminoglycan (sGAG) in medium and papain digests of agarose-chondrocyte constructs. Chondroitin sulphate [21] derived from the trachea of cows(Sigma, USA) was utilized as standard. The constructs total sGAG quantities were expressed as sGAG/DNA (µg/µg), while the liquid media's sGAG concentrations were expressed as mg/ml.

HYP (hydroxyproline) assay: The hydroxyproline (HYP) assay was performed on the papain digests and gathered media to ascertain the collagen content. Sigma's 4-hydroxyproline was utilized as the reference. In the literature, HYP: collagen converting factor is stated to be 1:8. HYP constructs content was expressed as HYP/DNA (µg/µg) and HYP concentrations [22] were reported as milligrams per mL (mg/ml) for liquid media.

MMP-13 (Matrix metalloproteinase-13) assay:Using the MMP-13 kit's methodology, the enzyme-linked immunosorbent assay (ELISA) (Cusa-bio) was used to measure the quantities of MMP-13 in cell culture medium. The concentrations of MMP-13 were

expressed in ng/mL [23].

4. Animals

The study used male Wistar rats, aged seven weeks, weighing between 200 and 250 g. The animals were kept in a polycarbonate cage at 23 ± 3 °C with a 12 h light/12 h dark photoperiod and given food and water at will without restriction. There were experiments conducted. in accordance with the institutionally approved guidelines for the use of laboratory animals PGP Life Sciences' animal ethics council (IAEC) is located in Hyderabad. (Approval No. PGP/OA/LS00129-042022/L3). Investigations of optimized formulations in Vivo. Test drug administration did not result in any aberrant symptoms or deaths during the study period (Table 4), nor did it cause any significant changes in body weight. Therefore, it was determined that the test substance did not induce weight gain shift or overarching symptoms [23,24].

4.1. Administration of F5 plus an monosodium iodoacetate (MIA) injection to induce OA

Rats were randomized at random into one of the following six groups: The groups included in the study were Normal (N) (saline injection, n = 6); Negative control (NC) (1.0 mg MIA injection, n = 6); M + F5-150 group (MIA (1.0 mg) + F5 (150 mg/kg/day, n = 6); M + F5-30 group (MIA (1.0 mg) + F5 (300 mg/kg/day, n = 6); the positive control was MIA (1.0 mg) + Celecoxib (100 mg/kg/day, n = 6). They used the baseline diet for ten days to acclimate. On day 10, 50 μL (60 mg/mL) of MIA (Sigma-Aldrich, MO, USA) was injected into the right knee joint using a 1 mL syringe in order to cause OA. Each experimental group received an oral dose of saline, 150 or 300 mg/kg F5, and 100 mg/kg Celecoxib (KekulePharma limited) following the injection of MIA, once a day for five weeks [25–27].

Percentage of the right hind paw bearing weight: On days 7, 13, 20, 27, and 35 following the administration of F5, changes in the weight distribution between the left and right hind feet were measured using an in capacitance tester (Columbus Instruments, 950 N. Hague Ave OH, USA). Rats were put in an angled glass container with a distinct location for each hind limb. onforce plate. The rats were given time to get used to the equipment, and while they were stationary, readings were noted. Each hind limb's downward force, expressed in grams, was evaluated and averaged over the course of 3 s (each data point represented the average of three readings). For the weight-bearing measures, the percentage of weight (in grams) carried on the right hind paw was calculated using the following formula [26].

$$\text{Percentage weight bearing} = \frac{\text{weight of right hind}}{\text{weight of left hind} + \text{weight of right hind}} \text{x}100 \quad (1)$$

4.2. Joint thickness measurement and evaluation of pain behaviour

An automated version of the von Frey hair evaluation process, the Electronic von Frey Aesthesiometer (IITC Life Science Inc., Victory Blvd Woodland Hills, CA), was used for nociceptive testing. The process was carried out prior to the injection of MIA (Day 9) and on specific days following the injection of MIA [28]. After F5 for 7,13,20,27, and 35 days management. The animals were put in an acrylic chamber with a metal mesh surface in a temperature-controlled room and given a 15-min break before to testing. Each animal had a touch stimulator positioned underneath it, and when the device was turned on, a thin plastic monofilament moved steadily forward and made contact with the paw's proximal metatarsal area. Starting below the detection threshold and gradually increasing until the stimulus became painful—as demonstrated by the removal of its paw, the filament applied a push to the plantar surface. An automatic recording of the force necessary to cause a paw withdrawal reaction was made in grams [29]. Following the administration of F5, the thickness of the knee joint was measured using digital callipers at predetermined weekly intervals.

5. Results and discussions

Rheumatoid arthritis and osteoarthritis are two inflammatory conditions for which *Uncaria tomentosa*, a traditional herbal remedy, is said to be effective. Consequently, the biomarkers were reported in the hydroalcholic extract utilizing major alkaloid-based biomarkers based on oxindole [10]. Thus, by lowering the dosage in modified formulations like FDOFs, which have higher systemic

Table 4
Percentage weight bearing on right hind paw.

% Weight-bearing post-F5 formulation administration in days					
	7	13	20	27	35
Normal (N)	51.33 ± 3.1	52.87 ± 3.7	49.12 ± 4.2	48.19 ± 3.8	51.57 ± 4.8
Negative Control (NC)	31.58 ± 2.4	29.64 ± 1.9	31.69 ± 2.8	30.25 ± 2.9	26.25 ± 3.9
MIA with F5 at 150 mg/kg/day (M + F5-150)	35.69 ± 2.9	32.69 ± 2.5	34.18 ± 2.1	36.58 ± 2.5	33.65 ± 4.2
MIA with F5 at 300 mg/kg/day (M + F5-300)	41.85 ± 3.6	39.12 ± 2.8	40.68 ± 3.6	41.69 ± 3.4	40.23 ± 3.4
MIA with Celecoxib at 100 mg/kg/day (PC)	43.95 ± 3.9	41.86 ± 2.3	44.25 ± 3.9	43.26 ± 3.3	41.93 ± 3.8

All the values are represented as Mean ± S. D (n = 3).

bioavailability, the plant's potential to reach the target location can be utilized. Less information about the conversion of *Uncaria tomentosa* extract into FDOFs that target osteoarthritis has been supported by the literature so far. Using natural film formers, synthetic polymers, super disintegrants, and plasticizers as independent variables and folding endurance and disintegration time as dependent variables via solvent casting method, the oral Fast dissolving films (FDOFs) were created using bark extract and optimized using the Box Behnken design protocol [26–30]. Studies demonstrating high rates of drug release in the optimized formulations, F5 and F13. (Tables 1–3). The *Uncaria tomentosa* extract in the formulated FDOFS has a sufficient dosage that demonstrates potential bioavailability as measured by drug release Further research on the pharmacokinetic estimation of drug in serum levels is a challenging task to demonstrate the F5 formulation as potential promising agent.

Tests for cell viability: After the chrondrocytes' cell viability was maximized, it was discovered that the treated groups' F5 and F13 cell counts were higher than those of the untreated cells. As a result, F5 and F13 increase cell viability (Fig. 2(a–c)) [31].

Effect of Fast dissolving oral films on

Assay for DNA content:The F5 and F13 formulations caused an increase in DNA concentrations from 7 to 35 days. The better state of the injured chondrocytes can be represented in the estimation [32] of enhanced DNA ($P < 0.0012$) (Fig. 3a).

GAG test, or glycosaminoglycan assay: The constructs' total sGAG amounts, which are reported as sGAG/DNA (μg/μg) and sGAG concentrations in the range of 25–30 μg/μg and 4–8 mg/mL, respectively, rescue for the damaged levels of IL-β treated chondrocytes (i.e., 26–40 μg/μg and 8–16 mg/mL), clearly explaining significant ($P < 0.001$) positive reforming potential of the F5 and F13 Fast dissolving oral films to preserve the cartilage, as these GAGs are important in the formation of chondrocytes (Fig. 3(b and c)).

HYP (hydroxyproline) assay: For the investigation, the papain-digested chrondrocytes [34] were employed. The effect was significant ($P < 0.001$) for the F5 and F13 treated groups, with HYP content elucidated as 0.6–0.8 HYP/DNA (μg/μg) and HYP concentrations of liquid media were reported as 9–12 mg per mL, respectively, clearly mentioning the osteoarthritic potential (Fig. 3(d and

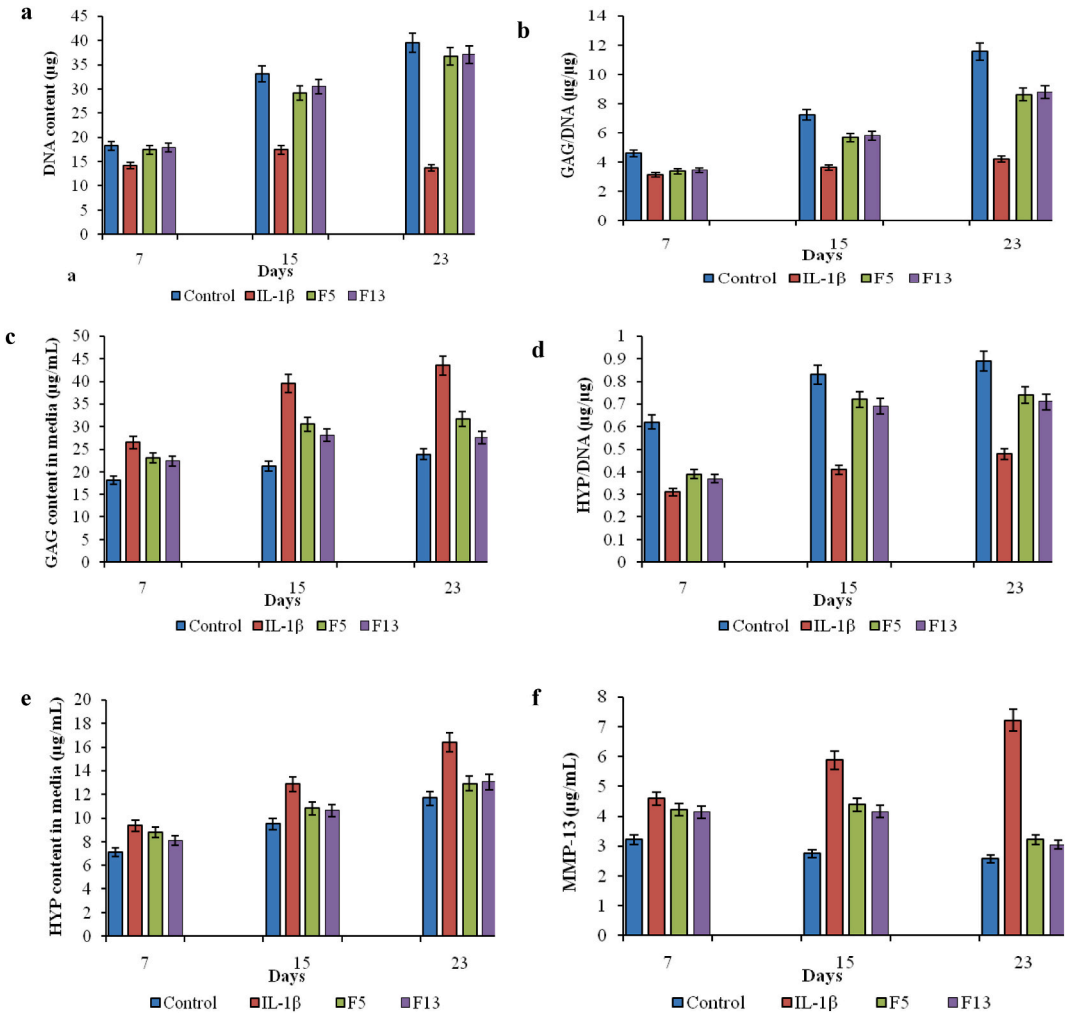

Fig. 3. (a) DNA content, (b) GAG content, (d) Hydroxyproline content of agarose-chondrocyte constructs; (c) GAG content, and (e) Hydroxyproline contents released into cell culture media, (f) MMP-13 levels in cell culture media (n = 3) at days 7, 15, and 23; Control: no-exposure to IL-1β; IL-1β: IL-1β added group; F5: IL-1β added and treated with F5-FDOF; F13: IL-1βadded and treated with F13-FDOF.

e)). HYP content indicated as 0.8–1 HYP/DNA (μg/μg) and HYP concentrations of liquid media were elucidated as 10–14 mg per mL for the treated groups.

MMP-13 (Matrix metalloproteinase-13) assay:MMP-13 concentrations [35] were expressed as ng/mL and were used to quantify the degree of arthritis in the cell culture. When compared to the non-treated groups, the treated groups' MMP-2 levels were observed to be reduced, suggesting that oral FDOFs have favourable arthritic potential (Fig. 3f). The findings were deemed significant ($P < 0.003$).

5.1. *In vivo studies of optimized formulations*

As OA progresses, there are concomitant structural changes and discomfort, necessitating complicated treatment [35]. Although the exact cause of the chronic pain response is unknown, it may be related to subchondral cortical and trabecular bone remodeling, articular cartilage degradation, inflammation of synovial joints, and a decrease in synovial fluid. Oxidative stress and inflammation in the disease are caused by the proinflammatory mediators, such as cytokines, which play a crucial role in the synovium. The thickness of the articular and subarticular bone decreases after three days of MIA-induced synovitis. The capacitance tester was used to determine the weight bearing capacity, and the Electronic von Frey Aesthesiometer (IITC Life Science Inc., Victory Blvd., Woodland Hills, CA) was used for the nosocomial testing (Fig. 4). Five groups were created from the seven group old mice (Tables 4–6) (Fig. 5). All groups received monosodium iodoacetate (MIA), with the exception of the control group. The study provided evidence of the unpleasant condition brought on by the MIA injection. After four weeks, the evoked condition was further evaluated in relation to time (Fig. 6 (a–c)). Additionally, the observation of damage to the knee joints as a result of weight or inflammation at the joints actively propels the mid- or early-stage ailment into a late-stage or chronic state. Early or moderate discomfort that gradually progresses to a chronic illness is an estimate.

The results of the study showed that both the inflammation and the joint's ability to support weight could benefit with restoration [28–33]. as 300 mg of F5 was administered instead of 150 mg, the weight bearing ability was dramatically recovered ($P < 0.0001$) as compared to 100 mg of celecoxib (standard). At greater medication dosages, there was a significant ($P < 0.005$) decrease in paw volume and inflammation. The extinguishing therapies are primarily focused on managing the significant pain associated with osteoarthritis. Therefore, there is currently a dearth of solid evidence supporting conventional medication therapy options. The current research study is quite fascinating which has formulated the herbal medicine *Uncaria tomentosa* extract into Fast dissolving oral films with reliable excipient and reported the positive significant result through invivo-invitro correlation studies at preclinical level. The FDOFs of U. tomentosa have addressed the demand for herbal therapy over synthetic with less toxicity and alleviation. Therefore, the oral fast-dissolving films of modified *U. tomentosa* extracts can significantly replace the usual dosage forms that cause side effects, highlighting the therapy aspect in osteoarthritis.

6. Conclusion

Osteoarthritis is the third most common condition affecting the elderly population. It causes significant pain, which can restrict mobility and cause joint dysfunction. Ultimately, this can lower quality of life. The most common remedy for this serious problem is oral fast-dissolving films. An alternative is enhanced herbal therapy. Traditionally recognized for its ability to treat osteoarthritis, the bark of *Uncaria tomentosa* was selected as the active ingredient (API) and transformed into oral dissolving films by QbD design, utilizing appropriate ratios of plasticizer, disintegrants, and hydrophilic film formers. Based on evaluation, the resulting FDOFs were found to be potent formulations; specifically, the drug release kinetics of F5 and F13 indicated that they were optimized. Using both in vitro and in vivo techniques, the osteoarthritis potential of the optimized formulations was further explored. In vitro techniques have demonstrated that the joint damage resulting from possible causal variables, such as decreased levels of GAGs, SGAGS, DNA, etc., was considerably mitigated when the F5 formulation was employed instead of the F13 formulation. As a result, the in vivo potential of the F5 formulation was further investigated utilizing models of MIA-induced osteoarthritis, where the restoration of the inflammatory

Fig. 4. Measuring weight-bearing capacity of hind paws over the treatment of test samples using in capacitance tester.

Table 5
Pain behaviour assessment.

Paw withdrawal threshold (gr). post-F5 administration in days					
	7	13	20	27	35
Normal (N)	22.14 ± 2.5	23.58 ± 2.7	29.37 ± 2.2	24.67 ± 1.4	28.76 ± 2.1
Negative Control (NC)	11.26 ± 1.0	9.58 ± 0.8	10.34 ± 0.5	9.47 ± 0.6	8.63 ± 0.7
MIA with F5 at 150 mg/kg/day (M + F5-150)	13.24 ± 1.1	11.47 ± 0.9	12.69 ± 1.3	13.69 ± 1.1	12.42 ± 1.1
MIA with F5 at 300 mg/kg/day (M + F5-300)	15.07 ± 1.2	17.93 ± 1.7	16.57 ± 0.9	17.93 ± 1.6	18.32 ± 1.6
MIA with Celecoxib at 100 mg/kg/day (PC)	16.98 ± 1.1	17.48 ± 2.1	14.93 ± 0.7	18.35 ± 1.5	17.82 ± 1.7

All the values are represented as Mean ± S. D (n = 3).

Table 6
Evaluatin of Knee joint thickness.

Knee joint thickness (mm)					
	7	13	20	27	35
Normal (N)	6.8 ± 0.5	7.8 ± 0.5	7.3 ± 0.5	8.1 ± 0.7	8.4 ± 0.8
Negative Control (NC)	10.87 ± 0.8	11.69 ± 0.9	11.42 ± 0.9	11.99 ± 0.8	13.08 ± 0.9
MIA with F5 at 150 mg/kg/day (M + F5-150)	9.68 ± 0.6	9.95 ± 0.8	9.89 ± 0.8	10.42 ± 0.6	10.67 ± 0.9
MIA with F5 at 300 mg/kg/day (M + F5-300)	9.12 ± 0.7	8.93 ± 0.7	9.46 ± 0.9	9.68 ± 0.7	9.78 ± 0.7
MIA with Celecoxib at 100 mg/kg/day (PC)	8.2 ± 0.6	8.64 ± 0.6	9.21 ± 0.7	9.21 ± 0.7	9.56 ± 0.6

All the values are represented as Mean ± S. D (n = 3).

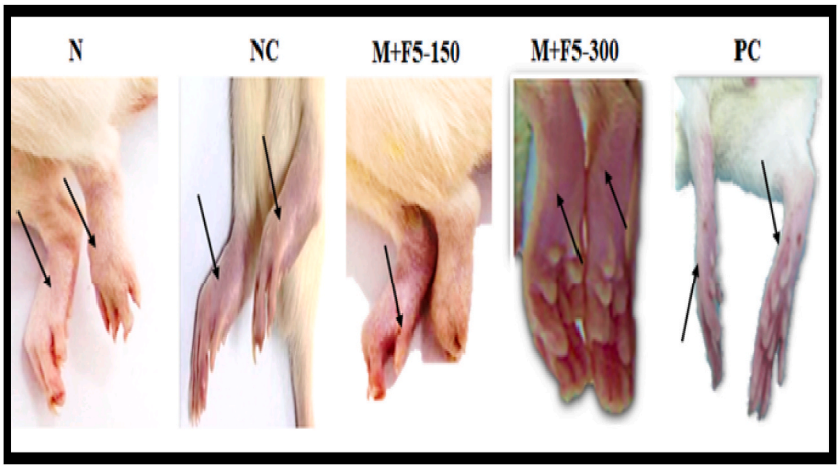

Fig. 5. Paw edema condition of various groups after administration of Test samples.

condition and weight bearing capacity were examined. Higher dosages of the F5 formulations significantly impact pain restoration and alsoadditionally, the joint's inflammatory state in comparison to the usual Celecoxib, demonstrating the use of complementary medicine in the osteoarthritis treatment. However, the pharmacokinetic profile in the clinical investigation was not elaborated, which is a shortcoming and might be of interest for future research. This formulation warrants further research in humans and may eventually be commercialized.

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Data availability statement

The requisite data will be made available on request.

Ethical consent

This current study was reviewed and approved by the Institutional Animal Ethical Committee (IAEC), PGP Life Sciences,

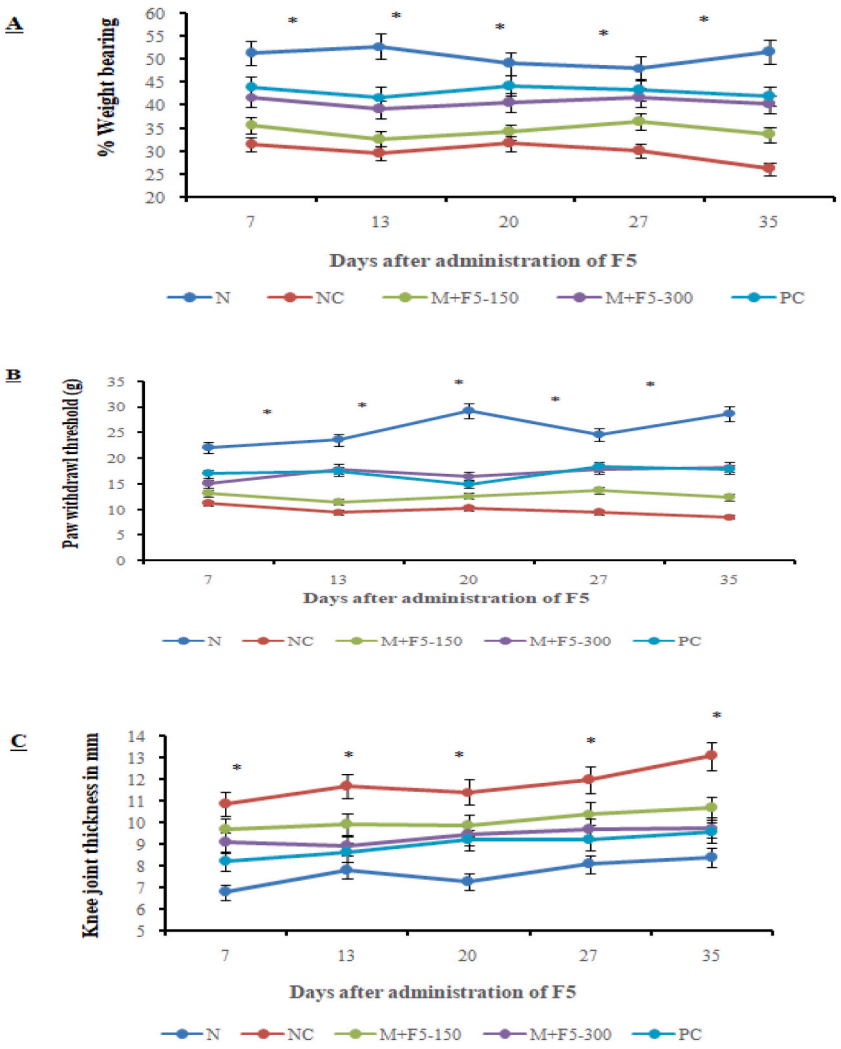

Fig. 6. Percent weight bearing (A), paw withdrawal threshold of the right hind paw (B), and knee joint thickness (C) of rats with monosodium iodoacetate-induced osteoarthritis. *Significantly different between normal and negative control ($p < 0.01$). Normal (saline, 20 mL/kg/day), negative control [monosodium iodoacetate (MIA), 20 mL/kg/day], M + F5-150 [F5@ 150 mg/kg/day, M + F5-300 F@300 mg/kg/day and positive control (PC, M + Celecoxib @100 mg/kg.

Hyderabad. (Approval No. PGP/OA/LS00129-042022/L3).

CRediT authorship contribution statement

Juluru Naga Sowjanya: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Proddoku Raja Rao:** Writing – review & editing, Supervision, Project administration, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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