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Research Article

In Vitro And In Vivo Approaches to Evaluate Uncaria Tomentosa Bark Extract Loaded FDOFS on Osteoarthritis Models

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Abstract

Osteoarthritis is one the leading health concerns worldwide affecting two third million with no proper treatment ensured to restore the normal function and completely relieving the joint pain. Oral fast dissolving films have promising action and targeted delivery with high drug loading capacity. The present investigation involves the study the invitro and in vivo activity of developed Oral fast dissolving films of U. tomentosa bark extract with optimized F5 and F13 formulations. For invitro evaluation a three-dimensional OA model was prepared with first passage chondrocytes grown on trypsin EDTA media in 1: 3 ratios. The OA agarose model was prepared with C20A4 chondrocytes on agarose gel (25 ± 5oC) in phosphate buffer solution. Cultivation of chondrocytes was done with 1 mL of RPMI-1640 (10% FBS) which was added with 20% (IL-1β) solution on third day of incubation and media was replaced time to time. The incubated cell line with 20,000 cells/well in 96-well plates were treated with 5 µL of 0.5% MTT reagent on fifth day of incubation and absorbance was measured at 570 nm. The effects were studied for 7, 13, 27, 35 days for the study effects of FDOFs on the cell lines were (Control, IL-1β, F5, and F13 treated IL-1β injected types). The chondrocytes in agarose constructs cultured only in media (RPMI-FBS) without IL-1b, served as control. The GAG, HYP and DNA quantitation analyses along with DNA content assay were performed to study the arthritic effect of optimized FDOF's i.e., F5. For in vivo studies Mon iodoacetate (MIA) induced arthritis models which is well established to understand weight bearing and response to tactile stimuli though the ongoing procedure is not known. The in vivo protocol was performed in seven-week-old male wistar rats with negative control of MIA and positive control as Celecoxib. The assessment of pain and thickness of the knee were estimated to be indicators of osteoarthritic potential. The study results revealed the F5 formulation has efficacy on the OA models which need a clinical investigation in humans.

Keywords: osteoarthritis; u tomentosa; assay; screening

Introduction

Osteoarthritis (OA) is a major challenging concern affecting millions of people and is known to be a chronic progressive disease of joints and surrounding tissue [1]. In fact, there is no complete treatment available with medicine so far for this degenerative disease of joints. Yet there are many reported long term treatments that can relive the pain or reduce the incidence of pain which include hyaluronan (HA) injection (Intraarticular). The disease is characterized by loss of joint cartilage in slow manner leading to deformity, dysfunction [2] and pain which were not overcome by the conventional oral drug treatments. Thus, there is surge in alternative treatments to address this issue by modifying the oral drugs with slow-release polymers that can retain the drug for prolonged time and release [3]. Moreover, the Uncaria tomentosa (Cats Claw) extract is widely known for treating the arthritis and inflammatory bowel disease [4]. The spiroindoline alkaloids present in the plant include mitraphylline, pteropodine are responsible for the antiinflammatory, apoptotic and play a major role in rheumatoid arthritis. The oral fast dissolving films have fastest market approach over the conventional tablets and improved patient compliance, thus considered to be the better strategy to overcome problems of osteoarthritis especially for pain management [5]. The API can be soluble or insoluble which when designed using suitable film forming polymers, plasticisers and disintegrants enhance the oral bioavailability, thus this novel approach [6] can be expanded to design even the herbal novel drug delivery systems with improved patient compliance and site specific and can be conveniently used in geriatrics. Pain is the key driving factor and restriction that imparts the treatment strategy to orient towards [7] the replacement surgery. The pain is categorized as two types initially a dull aching and later a shorter throbbing pain which comes frequently occurring in three stages (early, mid, progressive). The treatment protocol primarily involves lifestyle changes, NSAIDS, steroidal injections and

joint replacement therapy at chronic stage [8, 9]. A clear understanding of pain management to chronic is a better treatment option to be focussed on [10]. Thus, the evaluation protocols for osteoarthritis involve the determination of well-defined preclinical model of osteoarthritis such as invitro (pathogenic inflammatory mediators like a TNF-α, IL-6, and IL-1) and invivo protocols mediating the pain management strategy [11, 12].

The present investigation involved the development and evaluation of the novel fast oral dissolving films of Uncaria tomentosa bark extract using Box Behnken design [13, 26]. The evaluation and characterization [14] revealed the physicochemical nature and formulation parameters suitable for drug delivery. Further the in vitro and in vivo evaluation of efficacy of Uncaria tomentosa bark extract modified FDOFS was performed using various methods to prove that the product potential as a viable product for commercialisation and as alternative to existing product for the treatment of osteoarthritis.

Materials and Methods

All the chemicals, equipments and materials used for the experiment were of laboratory analytical grade with high purity. Equipments were sterilized prior to use. The statistical evaluation was done using ANNOVA [15].

Formulation of FDOF's by Qbd design and evaluationAbout 14 formulations were prepared by the Qbd Box Behnken design with the quadrate for optimization. Various natural film formers, synthetic polymers, super disintegrants, plasticizers were represented as independent variables with folding endurance and disintegration time as dependent variables using the solvent casting method. Further the formulation characteristics including physical and mechanical behaviour of films and drug release behaviour was evaluated. The 3D countour plots and response curves suing the design expert software were investigated which proved that the optimized oral dissolving films of extract with Pullalan gum, HPMC (polymers), Propylene glycol, PEG 400 (Co-solvents) and Croscarmellose sodium (superDisintegrant) are stable and uniform with formulation characteristics. The drug release rates prove that F5 and F13 formulations showed 99.90% drug release within 30 minutes following first order kinetics with satisfactory mechanical properties (Table 1,2,3). minutes following first order kinetics with satisfactory mechanical properties (Table 1,2,3).

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.																

Table 1: Composition of FODFs

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

F. code	Film forming capacity	Appearance of films	Tackiness	thickness (mm)	Surface pH	% moisture loss	% moisture gain
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 2: Characteristic formulation features of Uncaria tomentosa FODFs

. 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)					
n-vivo studies:					

Table 3: 33 factorial design Uncaria tomentosa FDOFS.

In-vitro Osteoarthritis evaluation

Culture and maintenance of C20A4 chondrocytes: Human Chondrocyte cell lines C20A4 were obtained from National Centre for Cell Sciences, Pune India. Chondrocytes were cultured in RPMI-1640 (10% fetal bovine serum (FBS), 10 U antibiotic/ml) (sigma Aldrich) medium at 37 oC under 5% CO2 in an incubator (5215, Shel Lab, USA). The growth medium was changed every third day and the chondrocytes were passage with trypsin-EDTA solution (0.05%) in a 1:3 ratio [16]. First passage chondrocytes were used for cell culture studies.

Establishment of three-dimensional in-vitro osteoarthritis model

For in-vitro experiments, a three-dimensional (3D) OA agarose model (Figure 1, 2)

***In-vitro* Osteoarthritis evaluation:**

1. Culture of Human Chondrocyte cell lines C20A4

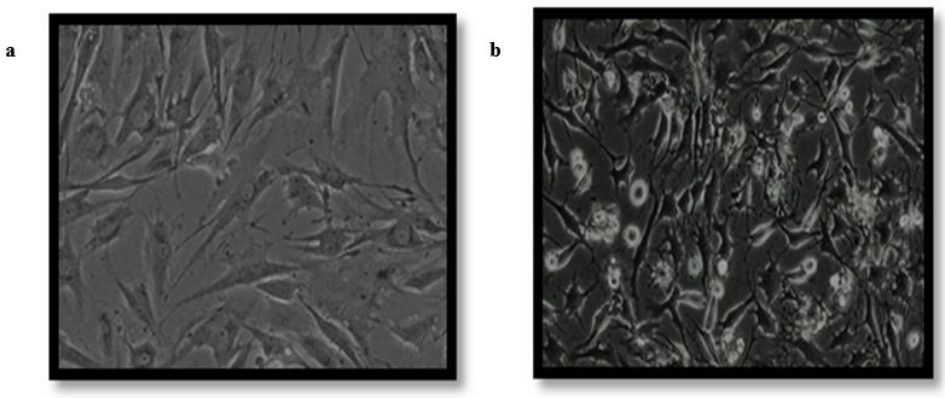

Figure 1: Culture of Human chondrocyte cell lines C20A4 in RPMI media without IL-1β treatment (a); With IL-1β treatment (b)

was developed using C20A4chondrocytes of OA. Briefly, C20A4chondrocytes [17] were embedded in agarose (2% low-melting agarose-gelling temperature 25 ± 5oC) (Sigma, USA) prepared in phosphate buffer solution. Then, equal volumes of chondrocyte suspension in double strength RPMI-1640 (20% FBS) were mixed with agarose to obtain a final chondrocyte concentration of 106 cells per mL in each well of a 24-well plate. About 1 mL of RPMI-1640 (10% FBS) was then added into each well. Medium was refreshed every 3 days. On the third day of in-vitro cultivation, 20 ng/mL of interleukin-1β (IL-1β) (Sigma-Aldrich, USA) was added into the medium. The same amount of IL-1β was added during each medium change.

Cell viability assays

The cell viability[18–20] of C20A4 chondrocytes, as well as IL-1β, induced OA model of C20A4 was assessed in contact with optimized formulations F5, and F13 at different concentrations(Figure 2)

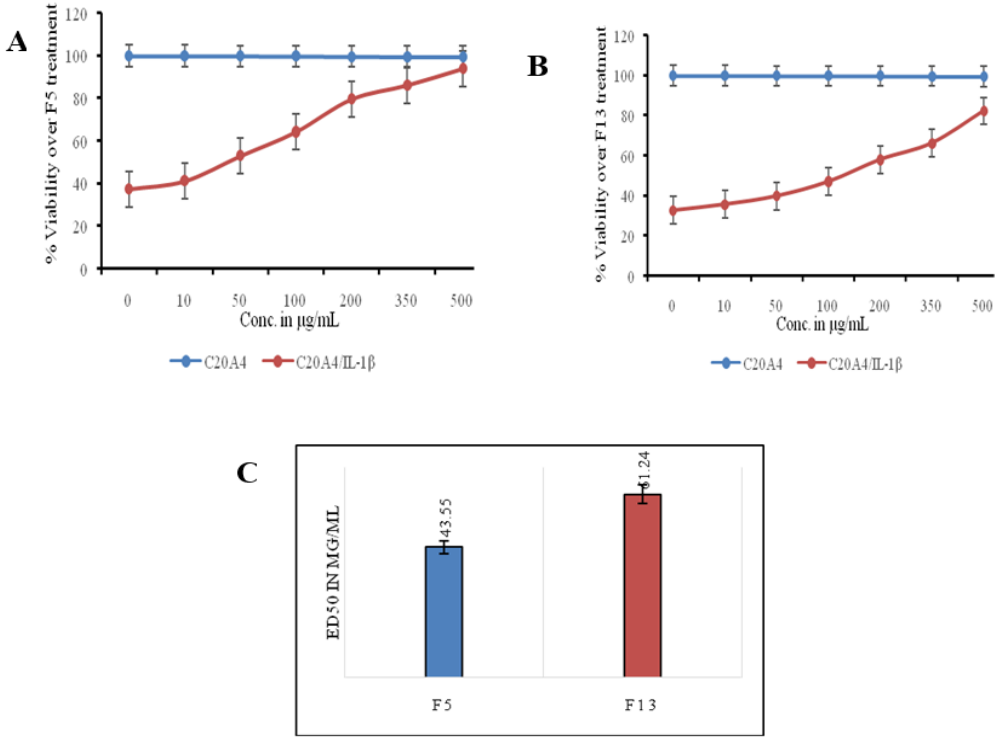

Figure 2: Cell viability assay of chrondrocytes with A) F5 treatment B) F13 treatment C) Effective growth

ranging from 0 to 500 µg/mL (0, 10, 50, 100, 200, 350, and 500 µg/mL. The C20A4, and C20A4/IL-1β cells were plated at a density of 20,000 cells/well in 96-well plates. C20A4/IL-1β cell were used after inducing OA for a period of 5 days of IL-1β treatment. After 24 h of culture incubation, F5 and F13 were suspended in PBS and incubated or 24 h at 37°C. 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 left for 3 h. All wells were incubated for 45 min with 1 µL of DMSO, and the absorbance was measured at 570 nm (8 points per well) on a Sunrise Tecan microplate reader.

Analysis protocol

Establishment of the in-vitro [21–23] OA model and effectiveness of the release systems were evaluated after incubations for 7, 15, and 23 days. For determination of the effects of FDOFs, the cell lines were divided into four groups 'i.e Control, IL-1β, F5, and F13 treated IL-1β injected cell lines. The chondrocytes in agarose constructs cultured only in media (RPMI-FBS) without IL-1b, served as control. The media were collected and stored at -80 oC for GAG, hydroxyproline (HYP) and MMP-13 analyses. Agarose constructs with chondrocytes were digested with papain for GAG, HYP and DNA quantitation analyses. The DNA amounts of chondrocytes in agarose were used to normalize the results of GAG and collagen amounts.

DNA content assay

DNA amounts of papain digested samples were determined by Hoechst 33258 dye (Invitrogen, Germany) with Fluorometer (Modulus, USA). Calf thymus DNA was used as standard. The measurement was performed according to the protocol of the instrument and DNA concentrations were measured as (µg) [20].

Glycosaminoglycan (GAG) assay

Total sulphated glycosaminoglycan (sGAG) amounts both in papain digests of agarose-chondrocyte constructs and in media were determined by using 1,9 dimethylmethylene blue (DMMB) assay. Chondroitin sulphate [21] from bovine trachea (Sigma, USA) was used as standard. Total sGAG amounts of constructs were reported as sGAG/DNA (µg/µg) and sGAG concentrations of liquid media were reported as mg/ml.

Hydroxyproline (HYP) assay: The papain digests and collected media were used for hydroxyproline (HYP) assay to determine the collagen content. 4-hydroxyproline (Sigma) was used as standard. HYP: collagen converting factor is reported as 1:8 in literature. HYP content of constructs was reported as HYP/DNA (µg/µg) and HYP concentrations [22] of liquid media were reported as mg per mL.

Matrix metalloproteinase-13 (MMP-13) assay

MMP-13 concentrations in cell culture media were quantified by enzyme-linked immunosorbent assay (ELISA) (Cusa-bio) according to the protocol of the MMP-13 kit. MMP-13 concentrations were measured as ng/ mL [23].

In vivo Studies of formulations

Animals: Seven-week-old male Wistar rats (200–250 gr) were used for this study. The animals were provided with food and water ad libitum and were housed in a polycarbonate cage at 23 ± 3°C under a 12 h light/12 h dark photoperiod. Experiments were performed according to the guidelines for the use of laboratory animals approved by the Institutional animal ethical committee (IAEC), PGP Life Sciences, Hyderabad. (Approval No. PGP/OA/LS00129-042022/L3). During the study period, there were no abnormal symptoms or deaths from the administration of the test substance (Table 4) and there were no significant changes in body weight, so it was concluded that the test substance did not cause weight change or general symptoms [23, 24].

% Weight-bearing post-F5 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)					

Table 4: Percent weight bearing on right hind paw

Induction of OA with Monosodium Iodoacetate(MIA) injection and administration with F5: Rats were randomly assigned to one of the six groups as follows: Normal (N) (injection of saline, n = 6); Negative control (NC) (injection of 1.0 mg MIA, 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); MIA (1.0 mg) + Celecoxib (100 mg/kg/day, n = 6, positive control). They acclimatized for ten days with the basal diet. On day 10, MIA (Sigma-Aldrich, MO, USA) was injected in a 1 mL syringe at a dose of 50 µL (60 mg/mL) in the right knee joint to induce OA. After injection of MIA, each experimental group was administered orally with either saline, 150 or 300 mg/kg F5, and 100 mg/kg Celecoxib (Kekule Pharma limited, Hyderabad) once daily for 5 weeks [25–27].

Percent weight bearing on right hind paw: Changes in weight distribution between the left and right hind paws were determined using in capacitance tester (Columbus Instruments, 950 N. Hague Ave OH, USA) on days 7, 13, 20, 27, and 35 after administration of F5.

Rats were placed in an angled plexiglass chamber so that each hind limb was positioned on a separate force plate. The rats were allowed to acclimate to the apparatus and when stationary, readings were taken. The downward force (measured in grams) applied by each hind limb was assessed and averaged over a three-second period (each data point was the average of three readings). For weight-bearing measurements [26], the percent weight (in grams) borne on the right hind paw was determined using the following formula

Percent weight bearing= [right hind weight/ (left hind weight + right hind weight)] x100

Assessment of pain behaviour and joint thickness measurement

Nociceptive testing was performed using an Electronic von Frey Aesthesiometer (IITC Life Science Inc. Victory Blvd Woodland Hills, CA), which is an automated version of the von Frey hair assessment procedure. The procedure was performed before the MIA injection (Day 9) and on given days after the MIA injection [28]. After 7, 13, 20, 27, and 35 days of F5 administration. The animals were placed on a metal mesh surface in an acrylic chamber in a temperature-controlled room and allowed to rest for 15 min before testing. A touch stimulator unit was oriented beneath each animal, and when the instrument was activated, a fine plastic monofilament advanced at a constant speed and touched the paw in the proximal metatarsal region. The filament exerted a gradual increasing force on the plantar surface, starting below the threshold of detection and increasing until the stimulus became painful, which was indicated by the removal of its paw. The force required to elicit a paw withdrawal reflex was recorded automatically and measured in g [29].

The thickness of the knee joint was measured at specified time intervals weekly using digital calipers after F5 administration.

Results and Discussion

The oral Fast dissolving films were synthesized by using the Uncaria tomentosa bark extract by standard Box Behnken design protocol for optimization using the natural film formers, synthetic polymers, super disintegrants, plasticizers [26–30] as independent variables with folding endurance and disintegration time as dependent variables by solvent casting method. The optimized formulations i.e F5 and F13 were proven by the studies which were found to have high drug release rates. (Table 1, 2,3).

Cell viability assays

the cell viability of the chrondrocytes was optimized and the treated groups with F5 anf F13 were found to be high in number than the untreated cells. Thus, the F5 and F13 enhance the cell viability (Fig. 1,2) [31]

Effect of Oral FDOF's on

DNA content assay

The DNA concentrations increased from 7 to 35 days under the influence of the F5 and F13 formulations. The estimation [32] of increased DNA ($P < 0.0012$) can be reflected with improved condition of the damaged chondrocytes (Fig. 2a).

Glycosaminoglycan (GAG) assay

The Total sGAG amounts of constructs reported as sGAG/DNA (µg/µg) and sGAG concentrations in the range of 25 to 30 µg/µg and 4–8 mg/ml respectively rescuing for the damaged levels IL-β treated chondrocytes i.e 26–40 µg/µg and 8–16 mg/ml clearly indicating significant ($P < 0.001$) positive reforming potential of the F5 and F13 FDOFs to retain the cartilage as these GAGs play [33] a major role in formation of chondrocytes (Fig. 2b, c).

Hydroxyproline (HYP) assay

The papain digested chondrocytes[34] were used for the study. HYP content reported as 0.8-1 HYP/DNA (µg/µg) and HYP concentrations of liquid media were reported as 10–14 mg per mL for treated groups and the effect was significant ($P < 0.001$) for the F5 and F13 treated groups ranging from HYP content reported 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 indicating the osteoarthritic potential (Fig. 2d, e).

Matrix metalloproteinase-13 (MMP-13) assay

MMP-13 concentrations [35] in the cell culture determine the level of arthritis and were measured as ng/ mL. The treated groups were found to have lowerMMP-2 levels when compared to the non-treated groups indicating the positive arthritic potential of oral FDOFs (Fig. 2e, f). The results were found to be significant ($P < 0.003$)

Invivo studies

The progression of the OA disease leads to the structural changes and pain associated which [36] leads to complex treatment. The chronic pain reflex is yet not clearly reported but may be associated with the damage of articular cartilage, inflammation of synovial joints, decrease in synovial fluid, joint damage, remodeling of subchondral cortical and trabecular bone. The key role of the proinflammatory mediators like cytokines in the synovium is responsible for the oxidative stress and inflammation in the disease. The MIA induced synovitis lasts for 3 days which is followed by decrease in the thickness of articular and subarticular bone.

The weight bearing capacity was measured using the capacitance tester and the Nociceptive testing was performed using an Electronic von Frey Aesthesiometer (IITC Life Science Inc. Victory Blvd Woodland Hills, CA) (Figure. 4).

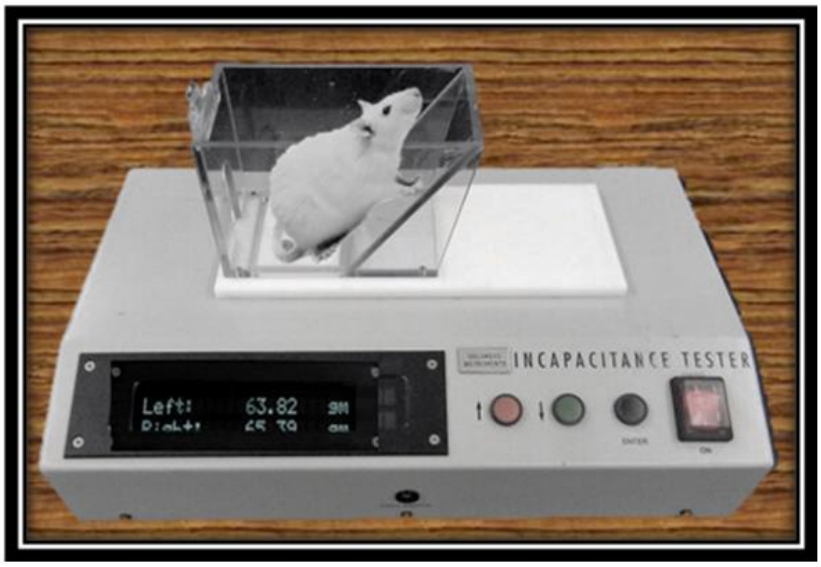

Figure 4: Measuring weight-bearing capacity of hind paws over the treatment of test samples using in capacitance tester

The seven group old mice (Table 5,6) were divided into five groups (Figure. 6).

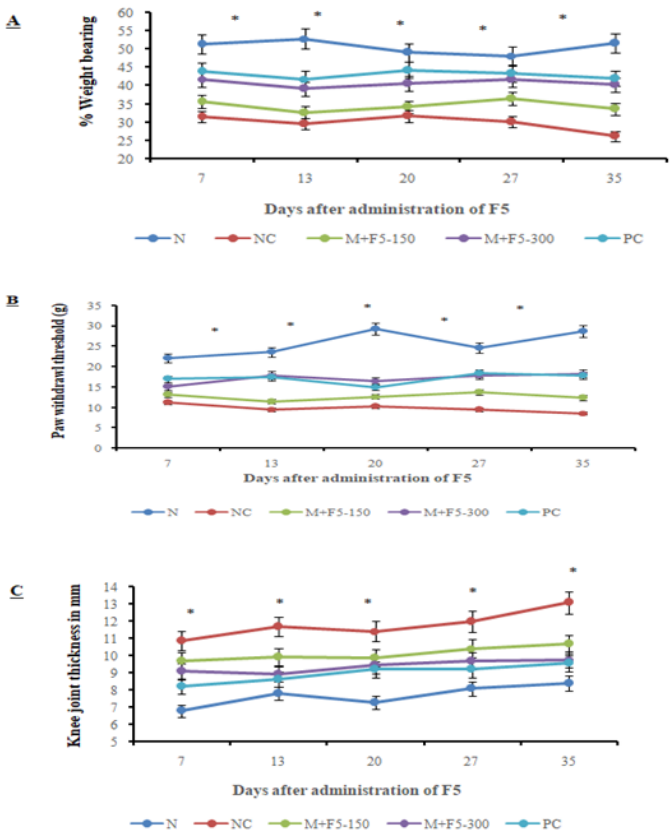

Figure 6: Percent weight bearing (A), paw withdrawal threshold of the right hind paw (B), and knee joint thickness (C) of rats with monosodium iodoacetate-induced osteoarthritis. *Significantly different between normal and negative control ($p < 0.01$). Normal (saline, 20 mL/kg/day), negative control [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

The monosodium iodoacetate (MIA) was given to all groups except the control group (Figure. 7).

The aversive condition due to the injection of MIA was demonstrated in the study. Further the evoked condition with reference to time after 4 weeks was observed (Figure. 6).

Further the observation of knee joint damage due to weight or inflammation at the joints actively driving the mid or early condition to late or chronic condition. The early or the midterm pain progressively reaching the chronic condition can be estimate.

Paw withdrawal threshold in 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 5: Assessment of pain behaviour

Knee joint thickness in 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)					
In-vitro Osteoarthritis evaluation:					
1. Culture of Human Chondrocyte cell lines C20A4					

Table 6: Assessment of Knee joint thickness

The study [28–33] results revealed a positive impact of restoration of the condition in terms of weight bearing capacity of the joint and also the inflammation condition. The weight bearing capacity was significantly restored with joint ($P < 0.0001$) with the dose of 300mg of F5 rather than the 150mg of F5 in comparison to 100 mg of the celecoxib(standard). The inflammation and reduction in the paw volume was significant ($P < 0.005$) in higher doses of the drug. Thus, the conventional dosage forms which elicit side effects can be substantially substitute with the oral fast dissolving films of U. tomentosa modified extracts indicating the treatment aspect in osteoarthritis.

Conclusion

Osteoarthritis is the third leading issue in geriatric population which has substantial pain leading to impairment, joint dysfunction and ultimately leading to the decrease in the quality of life. Thus, the significant issue has found the alternate with improved herbal therapy, the most popular addressable with oral fast dissolving films. The bark of Uncaria tomentosa is known traditionally for its osteoarthritic potential was chosen as the API and modified into oral dissolving films using suitable proportions of plasticizer, disintegrants and hydrophilic film formers by Qbd design. The resultant FDOFs were found to be potent formulation based on evaluation the F5 and F13 were found to be optimised based on drug release kinetics. The optimised formulations were further investigated for the osteoarthritis potential using in vitro and in vivo methods. The in vitro methods have proven that the damage being caused to the joint due to the potential causative factors like reduction in GAGs, SGAGS, DNA etc were significantly restored in presence of the formulation F5 rather than the F13 formulation. Thus, the F5 formulation was further investigate in vivo potential using MIA induced osteoarthritis models where the weight bearing capacity and inflammatory condition restoration was studied. The F5 formulations at higher doses has significant effect on restoration of pain and also the inflammatory condition of the joint compared to the standard Celecoxib indicating the Complimentary treatment medicine in the treatment of Osteoarthritis. This formulation has potential for commercialization after warranting investigation in the humans.

Declarations

Conflicts of interest

The authors declare no conflicts of interest

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Authors contributions

J. Naga Sowjanya has Conceived; Designed the experiments; Performed; Analyzed and interpreted the data; Scripted the paper. The guidance and supervision of the study was by Dr. Raja Rao along with the reagents, materials, analysis tools or data contribution.

Additional Declarations

No competing interests reported.

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