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Evaluation of topical formulations containing gingerol and piperine in rheumatoid arthritis

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Abstract
Background: Rheumatoid Arthritis (RA) is a chronic, inflammatory autoimmune disease causing synovial proliferation and joint destruction. Piperine and Gingerol have been reported to be effective on reduction of Tumor Necrosis Factor-TNF-α, key inflammatory mediator in RA and have an action on synovial fibroblasts.
Methods: Topical formulations (cream and emulgel) containing essential oils containing piperine and gingerol were prepared and evaluated. These formulations showed high retention (60-72%) at the site of action i.e. the dermis, where the fibroblasts are located, for a substantially long period which was demonstrated by *ex vivo* permeation studies. Drug was topically applied to the tibio-tarsal joint of the rats after 7th day of induction of rheumatoid arthritis using CFA. To evaluate the arthritic protective potential, the paw volume was measured up till 28th day, there was less % paw edema as measured by plethysmometer.
Results: The radiography and histopathology revealed lesser extent of bone and cartilage degradation in comparison to the untreated arthritic control.
Discussion: The amount of inflammatory cells in the synovial tissue, was highest in arthritic group, and was least in the test groups treated with the prepared formulations clearly indicating that both cream and emulgel were effective in controlling inflammation associated with arthritis.

Keywords: formulations, gingerol, piperine, rheumatoid

Introduction
Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease characterized by the presence of inflammatory mediators such as TNF α, IL-1, IL-6, which are known to aggravate the inflammation and bone destruction. [1] Disease modifying anti rheumatic drugs (DMARDs) along with non-steroidal anti-inflammatory drugs (NSAIDs) form mainstay of RA treatment [2, 3]. Many phytoconstituents such as Piperine and 6-Gingerol are reported to possess DMARD activity [4, 5, 6]. The activity has been attributed to inhibition of TNF α secretion and prevention of proliferation of synovial fibroblasts [7] Topical drug delivery of various anti-rheumatoid agents such as corticosteroids [8, 9], NSAIDs [10] has been widely reported, recently we have reported topical delivery of chloroquine [11, 12]. The logic behind this is that the fibroblasts which regulate the TNF α secretion and reside in dermal layers [13, 14] can be inhibited by topical administration and it saves the systemic load of the drug. The objective of present work is to formulate and evaluate topical formulations of phytoconstituents. Piperine and 6-Gingerol so as to achieve localization at site of inflammation thereby increasing the effectiveness of the therapy. The ginger oil and black pepper oil were formulated and evaluated as cream and emulgel apart from performance properties such as pH, spreadability the ex vivo permeation was studied and polyherbal topical formulations were demonstrated to exhibit significantly higher accumulation in dermal layers. The formulations were further studied in a CFA-induced pharmacodynamics model in rats and evaluated using X-ray, histopathology and paw edema. The topical treatment demonstrated a significant alteration in the progression of RA compared to standard treatment (chloroquine dispersion), bone destruction, joint space narrowing and infiltration of inflammatory cells were reduced to a much greater extent.

Materials
Black pepper oil and ginger oil were purchased from R.K Aroma, Mumbai. Cetyl alcohol, Sodium lauryl sulfate, Sodium carboxy methyl cellulose, Tween 80 and Span 80 were purchased from SD Fine chemicals, Mumbai; methyl and propyl paraben from Cosmo chemical, Pune.

Methods
Formulation of cream: The rHLB of oil blend (ginger oil : pepper oil) in ratio 5:8 was determined by emulsifying with various blends of Span 80 and Tween 80 at a concentration of 4%. The HLB of surfactant blend which yielded emulsion with least particle size and stability was taken as rHLB of oils [15].
The cream and emulgel was formulated according to the general formula given in table 1 [16]. Cetyl alcohol and glyceryl monostearate and ginger oil pepper oil blend were melted at 60^0C to form oil phase. Propy l paraben was added in the same phase. Sodium lauryl sulfate and methy l paraben was dissolved in water heated at 60^0C. The phases were stirred together till cream was formed (Make REMI model RQT-124 A).
For emulgel oil blend was mixed with Span 80 and added into

mixture containing water and Tween. This was then dispersed into sodium CMC gel base.

Table 1: Formulation Ingredients for Polyherbal Topical Formulations

Cream	Emulgel
Oil Blend	Oil Blend
Cetyl alcohol	Span 80
Sodium lauryl sulphate	Tween 80
Glyceryl monostearate	Sodium CMC
Water	Water

Evaluation of emulgel and cream
All formulations were observed for appearance, color and consistency.

pH Determination
pH of 1% dispersion of all formulations in distilled water was determined using pH meter (Make EI DELUXE 101).

Drug Content
% drug content of cream and emulgel was determined by HPTLC analysis (CAMAG). Ethanolic solutions of the formulations were spotted on the TLC plate and compared against the standard marker solutions piperine and gingerol respectively using mobile phase Toluene: Ethyl acetate (7:3) for development [17, 18, 19].

Globule size determination
The globule size analysis of formulations was performed using Malvern Zetasizer NanoZS90 (Malvern Instruments, Worcestershire, UK) and laser diffraction with beam length 2.40mm, range lens of 300 RF mm, and at 14.4% obscuration.

Viscosity
Brookfield Digital viscometer (RVDV Pro II, Brookfield Engineering Lab Inc) equipped with a ULC adapter and spindle S-92 was used to determine viscosity (cp) of the formulations. The viscosity was measured at 10 rpm after 30 seconds. Measurements were performed at ambient temperature and in triplicate.

Spreadability
The spreadability of the formulation was determined using apparatus Texture analyzer CEB Texture analyzer, Brookfield Engineering Labs, Inc., Model Texture Pro CT V1.4 Build 17.

***Ex vivo* permeation studies**
Rat skin was prepared by cleaning any soil from the surface with a mild skin cleanser, removing any hair with clippers and removing subdermal fat and fascia. The excised rat skin after hydrating in water for 1 hour was mounted on the Franz diffusion cell (Orchid Scientifics model FDC 03) with effective diffusion area 3.14 cm^2 and 7 ml cell volume with stratum corneum facing upwards [20] The receptor compartment was filled with phosphate buffer pH 7.4, and the assembly was maintained at 37ºC ± 0.5 under constant magnetic stirring. With reference to SUPAC (Scale-Up and Post approval Changes) guidelines laid by FDA [21], 300 mg of gel was applied to the membrane on the donor compartment and then covered with aluminum foil to prevent drying out. Aliquots were withdrawn at predetermined time intervals over a period of 8 hours and analyzed by HPTLC.

Pharmacodynamic Study
The pharmacodynamic study was carried out on male Wistar rats weighing 180-220 gm. The experimental protocol (Table 2) was approved by the Institutional Animal Ethical Committee constituted as per the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA/IAEC/PT-02/02-2K16).

Table 2: Experimental protocol for pharmacodynamic study

Group(n=6)	Treatment	Dose
Group I	Positive control	-
Group II	Arthritic control	-
Group III	Arthritic animals treated with standard	Solution of Chloroquine phosphate equivalent to 15 mg
Group IV	Arthritis animals treated with Cream	Applied topically to the joints
Group V	Arthritis animals treated with Emulgel	Applied topically to the joints

Induction of arthritis
Arthritis was induced by a single injection of 0.1ml Complete Freund's Adjuvant (CFA) containing heat killed and dried Mycobacterium tuberculosis into subplantar region of the right hind paw on day one [22, 23]. Animals were divided into five groups with six rats in each group. Treatment was initiated after the onset of arthritis (day 7) and continued once daily until the 28th day of the experiment. The standard group was treated with oral dose of chloroquine (dose equivalent to 15mg). To the test group IV cream was applied topically and test group V received emulgel treatment.

Evaluation of severity of arthritis
The primary lesions i.e. paw volumes of injected paws were measured on the day CFA was injected, using a digital plethysmometer. The paw volume was measured again on the 7^{th}, 14^{th}, and 21^{st} days after injection. During the experimental period, the body weight was measured using a digital weighing balance every third day after adjuvant injection. Animals were also observed for presence or absence of nodules in different organs like ear, fore paw, hind paw, nose and tail. Animals were scored '0' for absence and '1' for the presence of nodules.

Measurement of Hind paw volume
Paw volumes of both the right hind limbs were recorded from day 7 to 28 at 4-day interval using water displacement plethysmometer [24].

Radiological Analysis
On day 28, animals were anesthetized with anesthetic ether. Radiographs of the adjuvant injected hind paws were taken with X-ray instrument [24] The film focus distance was 60 inches and the machine was operated at 43kV peak, 2ma. The X-ray images of the adjuvant-injected limb of the rat were evaluated for radiographic changes and were graded as follows (Table 8).

1) Periosteal reaction (0-3; none, slight, moderate, marked)
2) Erosions
3) Joint space narrowing
4) Joint space destruction

Histopathology study**:** Morphological analysis of histological sections of knee joints is essential for quantifying the degree of joint damage and drug efficacy in the animal model. Rats were sacrificed on the 29th day of the study by ether anesthesia. Knee joints were removed and fixed for 4 days in 4% formaldehyde. After decalcification in 5% formic acid, the specimens were processed for paraffin embedding tissue sections and stained with haematoxylin and eosin [25].
Histopathological changes were scored using following parameters

- Infiltration of cells
- Bone erosion
- Joint space narrowing
- Connective tissue proliferation.
- Periosteal reaction

Stability Studies

Formulations were subjected to accelerated stability study at 40˚C 75% RH after 3 months. After completion of stability testing period, test samples were analyzed for appearance, particle size and pH. The stability data is presented in table 10.

Results

Selection of surfactant concentration range

Certain Emulsions containing various ratios of Tween 80 and Span 80 at a total blend concentration of 4% yielded emulsion which was found to be unstable as they rapidly creamed and produced unacceptable products (Table 3)

HLB Emulsifying Agent ≈ RHLB Oil Phase = Physically Stable Emulsion.

Stability Surfactant blends containing Span: Tween ratios of 1:9 and 2:8 respectively remained stable over a period of 3 days. However, the emulsion with the surfactant blend in the ratio 1:9 showed most stable particle size in the range of 250 nm. Therefore, surfactant blend in concentration range of 1:9 was selected. HLB of the oil blend was found to be 13.9

Table 3: Effect of emulsifier concentration on stability of emulsions

Surfactant blend (Span: Tween)	Average globule size (nm)*
9:1	Unacceptable product
8:2	Unacceptable product
6:4	Unacceptable product
4:6	Unacceptable product
2:8	450
1:9	259

Selection of the concentration of Cetyl alcohol -given in Table 4

Thus, formulation F2 which showed good PDI (Polydispersity index) and optimal particle size was selected and further used.

Table 4: Effect of Cetyl alcohol on Particle size and viscosity

Formulation	Particle size (nm)	PDI	Viscosity (CP)
F1	252	0.363	2480
F2	310	0.681	3050
F3	784.5	0.523	5940

Selection of concentration for gelling agent

Gels with varying concentrations of gelling agents (5% 7% 10%) were prepared and evaluated for viscosity. The gelling concentration showing optimum viscosity was selected (Table 5)

Table 5: Selection of Gelling agent

Concentration of gelling agent	Viscosity (cps)
5%	2680
7%	4210
10%	5780

Evaluation parameters for cream and emulgel

Table 6: Evaluation parameters for cream and emulgel

	pH	Particle size (nm)	Viscosity (cp)	Hardness (g)
Cream	7.02 ±0.15	310.1±1.75	3050	51
Emulgel	7.12±0.10	252.1±0.75	2500	36.6

***Ex vivo* Permeation Studies**

Table 7: % drug localized in skin

Formulation	% retained on the skin surface*	Cumulative % drug in receptor medium*	% localized in rat skin*
Cream	P-29.2 G-27.4	P-10.616 G-13.385	P-60.2 G-59.22
Emulgel	P-17.001 G-11.588	P-12.38 G-16.153	P-70.69 G-72.3
Oil Blend	P-30.722 G-34.591	P-4.466 G-3.018	P- 64.82 G-62.391

*n=3

Measurement of hind paw volume

Paw volumes of both the hind limbs were recorded at 4-day interval using plethysmometer. A significant reduction in paw volume was seen in cream treated group when compared to the arthritic control. Further, the reduction in paw volume in the emulgel treated group was highest when compared to the standard treated group (group III). Statistical analysis by two-way ANOVA followed by Bonferroni multiple comparison tests using Graph Pad Prism Software version

*P < 0.001, **P < 0.001 and ***P < 0.001 attributed all

values to be significant.

Table 8: X- Ray examination results: After treatment was initiated

Group	Degree of erosion	Jointspace narrowing	Bone destruction
Positive control	0	0	0
Arthritic control	++++	++++	++++
Test III Standard	+	++	0
Test IV	+	+	0
Test V	+	+	0

0: no abnormality detected
+: damage changes up to 25 %
++: damage changes up to 50 %
+++: damage changes up to 75 %
++++: damage changes more than 75 %

Radiological analysis

Radiographs taken post treatment and were compared for degree of erosion, joint space narrowing and bone destruction (Figure 1). The radiographic observations indicated that Test group V i.e. emulgel is more effective than the test group IV (Table 8). Degree of erosion was least for test group V and highest in the arthritic control. Marked bone erosion and joint destruction was seen in the untreated arthritic control group. Similarly test group IV and V showed less damage in terms of joint space narrowing and bone destruction when compared to test group III and the arthritic control group. Thus it was observed that the test group IV and V was the most effective in halting the bone erosion and destruction.

Table 9: Results of histopathology of joints

Group	Degree of erosion	Infiltration of cells	Connective tissue proliferation	Periosteal thickening
Positive control	0	0	0	0
Arthritic control	++++	++++	++++	++++
Test III Standard	++	+	+	+
Test IV (Cream)	+++	++	++	++
Test V (Emulgel)	+	+	+	+

0: no abnormality detected
+: damage changes up to 25 %
++: damage changes up to 50 %
+++: damage changes up to 75 %
++++: damage changes more than 75 %

Histopathology study

Results of histopathology of the joints of all 3 study groups are documented as analyzed by a pathologist (Figure 2). It was clearly observed that test group V (emulgel) gave more effective results than test group IV (cream). The degree of erosion was least and up to 25% for Test group V and up to 50% for Test group III while highest in the arthritic control group (Table 9). Similar results were obtained for connective tissue proliferation. Infiltration of cells which is expressed as the amount of inflammatory cells in the synovial tissue was highest in arthritic group, but test group V showed higher percent than Test groups IV clearly indicating that the test group V was effective in controlling inflammation associated with arthritis. Periosteal reaction was more marked in the arthritic control than both test groups.

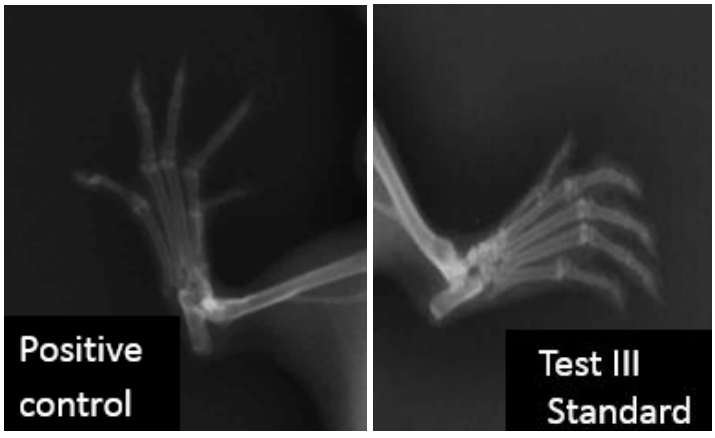

Fig 1: Radiographs taken after completion of study period

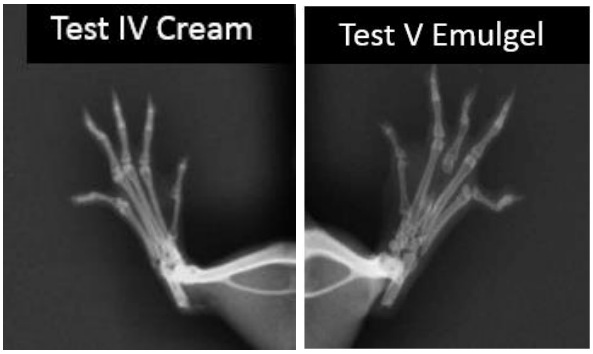

Fig 2: Results of Histopathology of joints

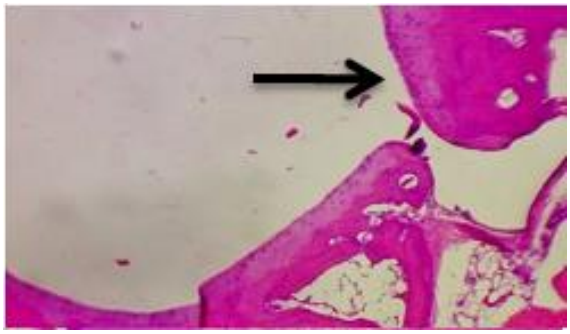

Fig 3

Positive Control– Area of joint. Note normal articular edges (arrow) no infiltration of inflammatory cells or any exudate in joint space. H&E 20X

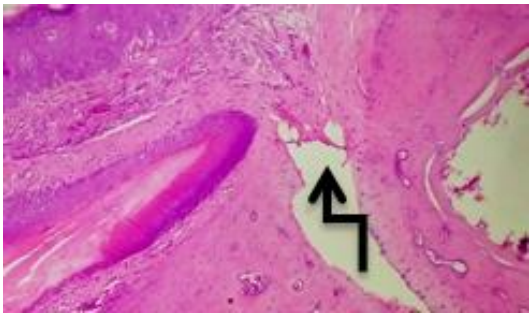

Fig 4

Test III Standard– Area of joint. Note minimal focal infiltration of Inflammatory cells and minimal exudate in joint space (arrow). H&E 20X

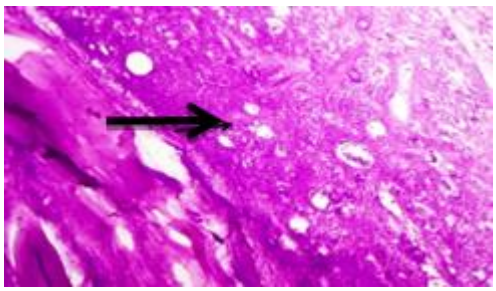

Fig 5

Negative control-Area of Joint. Note large infiltration of inflammatory cells (arrow) and exudate in joint space. H&E 20X

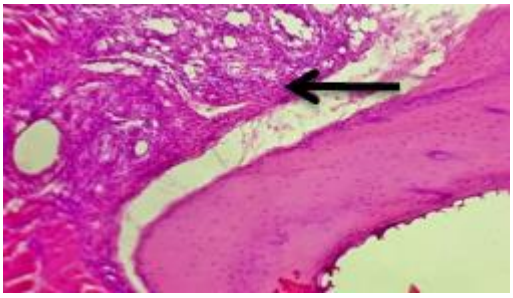

Fig 6

Test IV Cream– Area of joint. Note Mild infiltration of inflammatory cells (arrow) and minimal exudate in joint space. H&E 20X

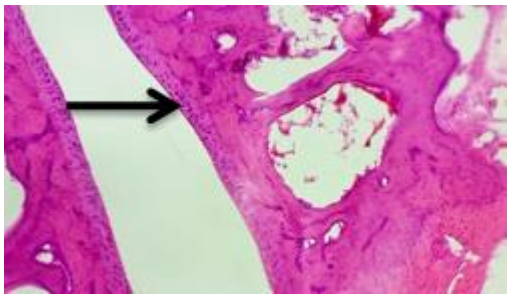

Fig 7

Test V Gel – Area of joint. Note no infiltration of inflammatory cells and no exudate in joint space. Articular edges of phalanges appear normal (arrow) H&E 20X

Stability Studies
The findings of the stability study is reported in table 10

Table 10: Stability data for cream and emulgel after 3 months

	pH	Viscosity (cp)	Particle size(nm)
Cream	7.36	3000	402.8
Emulgel	7.24	2400	375.8

Discussion
The data presented in table suggests that concentration of surfactant blend is unsuitable as it yields the unacceptable product. Therefore, surfactant blend in concentration ratio 1:9 was selected for further study of optimization.

pH Determination
The pH values of cream and emulgel were in the range of 7-7.1 (Table 6). Since the pH of formulations is neutral, hydration of the skin is facilitated that aids in permeation of the globules.

Drug Content
The drug content of the formulations was found to be 98.97% for piperine to 99% for 6-gingerol. Hence uniformity of drug content was found to be satisfactory. The quantity of the topical preparation weighed directly corresponds to the desired amount of active drug moiety i.e. drug on 'as is' basis.

Viscosity
Viscosity values for gels are totally attributable to the concentration of the gelling agent (Table 6). The emulgel being the combination of emulsions and gels show moderate viscosities and hence better acceptability. The values of viscosity measurements of all formulations are listed. Marketed preparation was evaluated for its viscosity to get a fair idea about the acceptable viscosity value which was found to be 2500 cps.

Spreadability
The observations for spreadability of all the formulations are listed in table 6. The spreadability of the formulations is a characteristic derived from its more basic property i.e. viscosity. The greater the viscosity, longer is the time taken for spreading. The gels are expected to spread easily on the skin areas when applied. The spreadability also depends on the polymer in formulation. In the study it was found that as changes in spreadability time are proportional to the changes in viscosity for different formulation.

***Ex vivo* permeation study**
Temperature control of the receptor fluid is crucial throughout the experiment and should be maintained at *in vivo* skin conditions (37ºC). As the rate and extent of skin absorption is temperature-dependent, the skin temperature should be maintained constant (37°C). The receptor fluid must be well mixed throughout the experiment. The viability of the skin is not a prerequisite for penetration testing, since the process depends on passive diffusion and not apparently on active transport. *Ex vivo* permeation study data is presented in table. The higher viscosity of the cream formulation can be said to be the reason for higher amount been retained on the skin. Since the globules were localized in the dermis, the % drug in receptor medium was to a lesser extent. The amount of drug remaining on the surface is that, that is either not yet released from the gel/cream base or un-diffused. The globules get entrapped in the dermal layer of skin from which the drug is released and the drug moiety is proposed to be acting on the fibroblasts residing in the dermis which plays a vital role in triggering inflammation and subsequent bone and cartilage damage. This is the rationale behind localizing the drug in the dermal layers of the skin.

Stability Study
After completion of stability testing period of three months, the cream and emulgel were evaluated for appearance, pH and particle size. It is evident that the formulation did not show any sign of instability and was stable over the testing period.

Conclusion
Topical preparations which would bypass gastrointestinal contact and exhibit a potential for reducing systemic toxicities were developed. Topical cream and emulgel system was developed and characterized. Formulation containing the essential oil blend showed highest retention of drug in the dermal layers of skin and hence is retained at the site of action i.e. the dermis, where the fibroblasts are located, for a substantially long period. The drug was topically applied to the tibiotarsal joint of the rats after the 7th day of induction of rheumatoid arthritis. To evaluate the arthritic protective potential, the paw volume was measured up till 28 days. It was observed that the emulgel formulation showed a greater protective potential than the cream. The radiographic studies and histopathology studies revealed lesser extent of bone and cartilage degradation in comparison to the non-treated arthritic control, standard oral solution. In addition the histopathology study confirmed that as the amount of inflammatory cells in the synovial tissue, was highest in the arthritic group, but showed the least percent in the Test groups IV and V clearly indicating that both cream and emulgel were effective in controlling inflammation associated with arthritis. Thus it was concluded that further development in the formulation prospects can yield a better product for clinical investigation.

Compliance with Ethical Standards
Conflict of interest
The authors report no declarations of interest

References

1. Marie-Christophe Boissier, *et al*. Rheumatoid arthritisFrom autoimmunity to synovitis and joint destruction Journal of Autoimmunity. 2012; 39:222-228.
2. Freedman A. Chloroquine and Rheumatoid Arthritis; A short-term controlled trial. Ann. rheum. 1956; 15:251-58.
3. Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF-α, IL-1β and IL-6 from lipopolysaccharide-stimulated human monocytes / macrophages by different modes. Rheumatology. 2006; 45:703-710.
4. Subhash Yende R, *et al*. Antirheumatoid activity of aqueous extract of *piper longum* on freunds adjuvant-

induced arthritis in rats. Indian journal of pharmaceutical and scientific research. 2010; 1(9):129-133.
5. Yasuka Isa, *et al.* 6-Shogaol and 6-gingerol, the pungent of ginger, inhibit TNF-a mediated downregulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes. Biochemical and Biophysical Research Communications. 2008, 429-434.
6. Bang JS, Oh DH, Choi HM, Sur BJ, Lim SJ, Yang JY, Yoo MC, *et al*. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1β-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis Research & Therapy. 2009; R49.
7. Huber LC. Synovial fibroblasts: key players in rheumatoid arthritis. Rheumatology. 2006; 45:669-675
8. Maia CS, Mehnert W, Schafer-Korting M. Solid lipid nanoparticles as drug carriers for topical glucocorticoids. Int J Pharm. 2000; 96:165-7.
9. Jan H. Vaile and Paul Davi Topical NSAIDs for Musculoskeletal Conditions Drugs. 1998; 56(5):783-799
10. Molin S, Abeck D, Guilabert A and Bellosta M Mometasone Furoate: A Well-Established Topical Corticosteroid now with Improved Galenic Formulations. J Clin Exp Dermatol Res. 2013, 4:3.
11. Bhalekar *et al.* Anti-rheumatic activity of chloroquine-SLN gel on wistar rats using complete freund's adjuvant (CFA) model. Indian Journal of Rheumatology. 2015; 10:58-64.
12. Bhalekar MR, Upadhaya PG, Madgulkar AR. Fabrication and efficacy of Chloroquine nanoparticles in CFA-induced arthritic rats using TNF-α ELISA kit. European Journal of Pharmaceutical Science. 2016; 84:1-8.
13. Dimitris Kontoyiannis, George Kollias. Fibroblast biology Synovial fibroblasts in rheumatoid arthritis: leading role or chorus line. Arthritis Research & Therapy. 2000; doi: 10.1186/ar109
14. Beatrix Bartok, Gary S. Firestein. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. Immunol Rev. 2010; doi: 10.1111/j.0105-2896.2009.00859.x
15. Lara Orafidiya O. Oladimeji FA. Determination of the required HLB values of some essential oils. International Journal of Pharmaceutics. 2002; 241-249.
16. Harry Ralph. Harry's Cosmeticology'. The principles & practices of modern cosmetics. Sixth edition, 54-55.
17. Shrimanker Mitali, *et al*. Hptlc Quantification Method of Piperine and Zingiberene in A Veternary Polyherbal Formulation. International Journal of Current Research. 2012; 4(12):477-479.
18. Jain PS, Tatiya AU, Bagul SA, Surana SJ. Development and Validation of a Method for Densitometric Analysis of 6-Gingerol in Herbal extracts and Polyherbal Formulation. J Anal Bioanal Techniques.2011, 2:4
19. Iram Rakhshi1, Pawar RK, Singh KC. Development of Hptlc Method for the Determination of Piperine in Chitrak Haritaki An Ayurvedic Formulation. Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 2015; 3(3):95 -102.
20. Bhalekar MR, Upadhaya PG, Nalawade SD, Madgulkar AR, Kshirsagar SJ. Anti-rheumatic activity of Chloroquine-SLN gel on wistar rats using complete Freund's adjuvant (CFA) model. Ind j rheumatol, 2015.
21. Guidance for industry nonsterile semisolid dosage forms. Scale-Up and Post approval Changes (SUPAC): Chemistry, manufacturing, and controls; *in-vitro* release testing and *in-Vivo* bioequivalence documentation.
22. Snekhalatha U, Anburajan M, Venkatraman B, Menaka M. Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Zeitschrift für Rheumatologie. 2012; 1-7.
23. Tsai CC, Lin CC. Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on carrageenan- and adjuvant-induced paw edema in rats. Journal of Ethnopharmacology. 1998; 64(1):85-89.
24. Ekambaram S, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of *Strychnos potatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement. Altern. Med. 2010; 10:56.
25. Eseyin O, Ebong A, Ekarika J, Udo I. Changes in some pharmacokinetic parameters of chloroquine by Gnetum Africana. Maced. J Med. Sci. 2012; 5(3):275-279.