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Characterization evaluation of chitosan loaded transdermal film of Benincasa Hispida (Thunb.) Cogn. for the treatment of arthritis

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***Abstract*---**Hence, in the present study, the plant of *Benincasa hispida* (Thunb.) Cogn. have been selected for anti-arthritic activity in rats. The films can be made with a constant drug content and little batch variability using the method used. Spectrophotometric analysis at 260 nm was used to determine drug concentration. Drug concentration ranges from 2.37 mg/cm^2 to 2.48 mg/cm^2 across all formulations, suggesting reasonable consistency. The surface morphology of the film was analyzed by scanning electron microscopy, and the results validated the film's smooth, glossy, and aesthetically attractive look. An indicator of Benincasa hispida's anti-arthritic activity at doses of 200&400 mg/kg, po, is paw swelling. Increasing SA content resulted in a larger film viscosity (measuring between 392.13 and 403.45 Cp).

***Keywords*---**Benincasa hispida (Thunb.) Cogn., formulation, anti-arthritic activity.

Introduction

Rheumatoid arthritis is a long-term auto-immune multi-system sickness characterized by the immune system's erroneous assault on healthy tissues and joints, resulting in inflammatory synovitis and the eventual degeneration of joint ankylosis and articular cartilage. In accordance with the accepted definition of the

term diseases of the autoimmune kind are characterized by a negative response to the body's own immune system. The immune system is the body's built-in defensive mechanism, a collection of cells and antibodies whose mission it is to "seek and destroy" any foreign substances that may pose a threat. Synovium is a thin, fragile membrane that provides nutrition for cartilage, which becomes thickened and painful in rheumatoid arthritis. Moreover, the structural structure of the synovial interstitium is composed of collagens, fibronectin, and hyaluronic acid, all of which are produced by synovial cells, making the joints lubricated and facilitating easy movement [2-4].

Methodology

Formulation and evaluation of chitosan loaded transdermal films

Extracted chitosan-loaded transdermal films from Benincasa hispida (Thunb.) Cogn. were made by a mold-and-solvent-casting procedure in this work. A 25cm^2 mold was made with a 5cm length and 5cm breadth [5].

Preparation of extract-loaded transdermal films

We used a solvent casting process to make the transdermal films. Aluminum foil was stretched to form a membrane that would act as a backing for the mold. In order to make films utilizing plant extract, the solution casting method was utilized. The required amount of LBG will be measured out, and then a polymeric solution will be prepared with the right amount of water and let to sit for 2 hours after being stirred. Plant extract (2.5 mg/mm^2) and menthol (3% w/w) were dissolved in ethanol (6 mL) by stirring for 10 minutes. Glycerin concentrations ranging from 1% by weight to 5% by weight were added to the aforementioned mixture and stirred for 30 minutes to produce polymeric solutions. The wet substance was then packed into transparent plastic-lined glass molds and dried in a vacuum drier before the membrane was removed. Cast polymer films from each group will be removed, wrapped in aluminum foil, and stored in a desiccator until additional analysis may be conducted [6].

Evaluation of chitosan loaded transdermal films

Overall uniformity, drug content, anti-arthritis efficacy, film thickness, tensile strength, percentage elongation, folding durability, moisture absorption, moisture loss, and moisture gain were all evaluated [7].

Uniformity of weight

To ensure that each batch of film had the same weight, we weighed three samples from each batch. Individual weights were monitored to ensure they were within a reasonable range of the group average. To prepare the films for testing, they were dried at 40 °C for 3 hours [8].

Uniformity of film thickness

Screw gauge measurements of film thickness were taken at many locations [9].

Three separate readings were taken from various locations on each film and averaged to obtain the results.

Tensile strength

Hounse field universal testing machines were used to measure the films' tensile strength. It had two grips, the top one being adjustable and the bottom one being stationary. The test films were held in place between the load grips, and force was applied progressively until the films broke at a predetermined size. Newtons were used to directly measure the tensile strength of the films [10].

Tensile strength = Tensile load at break / Cross sectional area

Percentage elongation of the films

Extended duration as a percentage of the original length is a measure of how much a film has been extended. How far a specimen can be stretched before breaking is shown. The Hounse field universal testing machine is used for this purpose [11].

%Elongation = Maximum length recorded at break – Original length × 100 / Original length

Folding endurance

Durability of folding was evaluated by hand for the finished films. Consistent cuts were made at two centimeter intervals, and the film was folded repeatedly at the same point until it broke [12]. It was possible to quantify the film's folding endurance by measuring the number of times it could be folded in the same spot before snapping.

Percentage moisture absorption

Numerous films' hygroscopicity was tested at a relative humidity of 80%. (RH). It was decided to take 1cm2 films from each batch. To preserve their quality, the films were properly weighed before being stored in a desiccator with 100 ml of fused aluminum chloride, which keeps relative humidity at 80%. The films were weighed after being stored for 3 days [13]. A formula was developed to determine the relative humidity retention.

% Moisture absorption = Final weight – Initial weight × 100 / Initial weight

Percentage moisture Loss

Reduced to $1cm^2$ films, all batches were eliminated. After being weighed precisely, the films were placed in a desiccator with some anhydrous calcium chloride. After 3 days in storage, the films were weighed.

% Moisture loss = Initial weight – Final weight × 100 / Initial weight

Drug content

Cut-off sections of a prepared film with an area of 1cm^2 were individually weighed. To a 100 ml volumetric flask, the films were deposited, and to the flask, 100 ml of phosphate buffer solution (pH 7.4) was added. Twelve hours were spent agitating the material using a magnetic stirrer. Whatmann filter paper was used to strain the contents [14]. The drug concentration of the filtrate was determined by comparing it to a reference solution made up of just placebo films using spectrophotometry at 260 nm.

Scanning Electron Microscopy

In order to conduct scanning electron microscopy (SEM) research, a JOEL JSM-T330A is used [14]. Drug distribution in the film was determined by analyzing the SEM images of Formulation F3 and its exterior morphology.

Stability of the transdermal films

Over the course of 90 days, the drug content of all of the aforementioned formulations was checked at 25, 30, and 40 degrees Celsius with 60, 65, and 75 percent relative humidity, respectively[15].

In vitro Drug Diffusion Study

These tests on drug diffusion were performed in a glass tube specifically designed for that purpose. Two and a half centimeters of film was put in a receptor compartment filled with buffer solution at one end of an open glass tube. An electric magnetic stirrer, rotating at 100 revolutions per minute, was used for the assembly process. A constant temperature of 37.1 degrees Celsius was maintained throughout the system. At predetermined time intervals, a constant volume of receptor media (buffer) was removed, and this volume was replaced with fresh saline [16]. Samples of various drug concentrations were spectrophotometrically measured at 260 nm and compared to a blank.

In-Vivo Anti-Arthritic Study [17]

Chemicals

The study employed Freund's complete adjuvant (Sigma Aldrich), and all other substances and reagents were of analytical grade and acquired from reliable sources.

Animals

Male Wistar rats weighing between 150 and 200 g were used in the study. The animals were kept in standard enclosures and had access to standard pellet food and water at all times. The Institutional Animal Care and Use Committee (IACUC) gave final approval to all experimental procedures involving animals, and all procedures were carried out in compliance with CPCSEA guidelines (IAEC) CPCSEA/IAEC/JLS/17/03/22/037.

Induction of arthritis

In order to induce arthritis in mice, researchers injected 0.2 ml of Complete Freund's adjuvant dissolved in mineral oil (sterile) into the sub-plantar region of the hind paw. When the animals first arrived, they were anesthetized with a little dose of ether vapor.

Treatment regimen

Anti-arthritic activity was measured using the method described by Jubie et al. Animals were sorted into groups according to their weight, then labeled and housed in individual cages with letters to serve as identification. Each of the five groups of animals consisted of six specimens. Following is the dosing plan that was used for each group:

1. Group I -Vehicle control, 1% w/v DMSO, p.o; (nonarthritic);
2. Group II - Negative control (0.1ml Complete Freunds adjuvant);
3. Group III -Arthritic animals treated with standard, 0.75 mg/kg Methotrexate, p.o;
4. Group IV - Arthritic animals treated with 200 mg/kg of *Benincasa hispida* (Thunb.) Cogn., p.o;
5. Group V -Arthritic animals treated with 400 mg/kg of *Benincasa hispida* (Thunb.) Cogn., p.o;

Daily administration of a DMSO solution at 1% w/v was made to the vehicle control animals. On day 1, while under mild ether sedation, the control group received an injection of 0.2 cc Complete Freund's adjuvant in mineral oil into the sub-plantar region of the hind paw. The stock solution for low-dose, high-dose, and standard-dose animal treatments was generated daily based on the animals' body weights. Given its solubilizing action and insolubility in water, the plant extract was diluted in DMSO. Oral cannulas were used to administer the newly produced medication to the animal group. Each group received 40 more days of medication therapy.

Every day, the damaged paw and overall health of the animal was checked properly. Paw volume, body weight, the arthritic score, and behavioral data like locomotor activity all contributed to the health state parameter. Each animal was weighed once a week using a standard single-pan weighing scale, and its average body weight was recorded in grams. For this study, we tracked how long it took each animal to cover a distance of two meters, and then used statistical methods to draw conclusions about the animals' speed and agility. On day 41, the animals were killed so the histology of the joint could be examined.

Parameters

1. Paw edema volume
2. Arthritic score
3. Locomotor activity
4. Body weight

Statistical Analysis

Mean SD was used to examine the data (SEM). Statistical comparisons between groups of research subjects were performed using one-way and two-way analysis of variance (ANOVA) as well as Dunnett's test (for post hoc analysis). Statistically, the results are trustworthy since the P 0.05 significance level was reached. To analyze the data, we utilized GraphPad Prism.

Results and Discussion

Formulation and evaluation of chitosan loaded transdermal films

In this experiment, we used a mold and solvent casting technique to create Benincasa hispida (Thunb.) Cogn. extract chitosan laden transdermal films. A 25cm^2 mold was made with dimensions of 5cm in length and 5cm in breadth.

Table 1. Formulation chart of *Benincasa hispida* (Thunb.) Cogn. transdermal films

Formulation code	*Benincasa hispida* (Thunb.) Cogn. (%)	Locust bean gum (%)	Sodium alginate (%)	Glycerin (%)	Menthol (%)
F1	2.5	3.0	90.0	0.5	1.0
F2	2.5	24.5	68.0	1.0	2.0
F3	2.5	38.5	50.0	2.0	3.0
F4	2.5	28.5	58.0	3.0	4.0
F5	2.5	9.5	78.0	3.5	4.5”

Evaluation of Transdermal films

Uniformity of weight

This average weight was determined by weighing three separate films from each batch. Digital balances were used to determine the final weight of the dry films. Both of the films were of similar density.

Table 2: Uniformity of weight of *Benincasa hispida* (Thunb.) Cogn. extract loaded films (2.5 mg/cm^2)

S. No.	Formulation code	Weight of the patch in mg			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	F1	162	164	167	164.31±2.06
2.	F2	165	169	168	167.65± 1.51
3.	F3	197	195	193	194.31± 1.51
4.	F4	188	191	193	190.65± 1.13
5.	F5	182	185	180	182.65± 3.49

*Standard deviation, n=3

Uniformity of film thickness

The thickness of the films was measured at different points using screw gauge.

Table 3: Uniformity of thickness of *Benincasa hispida* (Thunb.) Cogn. extract film (2.5 mg/cm^2)

S. No.	Formulation code	Thickness of the patch in mm			Mean±S.D*
		Trial I	Trial II	Trial III	
1.	F1	0.10	0.12	0.14	0.12±0.02
2.	F2	0.11	0.14	0.14	0.12± 0.01
3.	F3	0.12	0.14	0.15	0.13± 0.01
4.	F4	0.14	0.12	0.14	0.13± 0.01
5.	F5	0.15	0.14	0.15	0.14± 0.01

*Standard deviation, n=3

Tensile strength

The films' tensile strength was measured using a Hounse field universal testing equipment. We utilized the dial's reading in Newton's to calculate the tensile strength of the films. There is enough tensile strength and elongation at break in the films. In contrast to the reduction in elongation as a percentage, tensile strength has risen.

Percentage elongation

The percentage elongation of films indicates the maximum allowable extension of a specimen before it breaks. The Hounsei field universal testing machine was used for the examination.

Table 4: Tensile strength and % elongation of *Benincasa hispida* (Thunb.) Cogn. extract film (2.5 mg/cm^2)

S. No.	Formulation code	Tensile strength (N/mm^2)	Percentage elongation
1.	F1	2.41± 0.01	19.28± 0.61
2.	F2	2.51± 0.03	19.54± 0.21
3.	F3	2.65± 0.02	20.43± 0.29
4.	F4	2.84± 0.02	22.38± 0.12
5.	F5	3.06± 0.13	24.36± 0.26

Folding endurance

For the ready films, we measured the folding endurance by hand. After cutting a strip of film 2 cm by 2 cm to the exact dimensions, we folded it over and over again in the same spot until it finally snapped. Film's ability to withstand repeated folding without ripping was quantified by measuring the number of times it could be folded in the same location.

Table 5: Folding endurance of *Benincasa hispida* (Thunb.) Cogn. extract loaded film (2.5 mg/cm^2)

S. No.	Formulation code	Folding Endurance			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	F1	244	234	233	235.33± 3.84
2.	F2	244	252	244	246.67± 5.00
3.	F3	262	263	270	266.67± 2.14
4.	F4	264	274	260	265.33± 3.01
5.	F5	271	269	269	266.33± 5.35

*Standard deviation, n=3

Percentage moisture absorption

The relative humidity in these moisture absorption tests was set at 80%. Moisture absorption percentages were low across the board for the films.

Percentage moisture Loss

Desiccant with anhydrous calcium chloride was used in the moisture loss trials. The proportion of moisture lost was lowest across all the films.

Table 6: Data of percentage moisture absorption and moisture loss (2.5mg/cm^2)

S. No.	Formulation code	% Moisture Absorption Mean ± S.D*	% Moisture Loss Mean ± S.D
1.	F1	1.59± 0.32	1.21± 0.13
2.	F2	1.58± 0.40	1.04± 0.08
3.	F3	1.61± 0.62	0.96± 0.14
4.	F4	1.36± 0.39	0.89± 0.08
5.	F5	1.85± 0.36	0.80± 0.37

*Standard deviation, n=3

Drug content

Little fluctuation in medicine content occurred between batches, and the formulations were generally constant.

Table 7: Drug content uniformity and percentage of drug content (2.5 mg/cm^2)

S. No.	Formulation code	Drug content in mg	% of drug content
1.	F1	2.35	93.51
2.	F2	2.38	94.14
3.	F3	2.46	96.50
4.	F4	2.40	9460
5.	F5	2.39	93.76

Scanning Electron Microscopy (SEM)

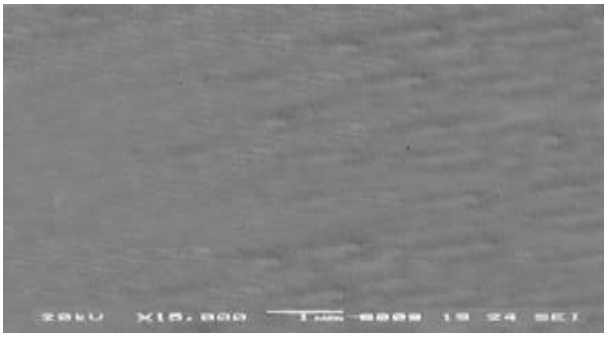

Figure 1: Scanning electron microscopy of *Benincasa hispida* (Thunb.) Cogn. extract loaded films

Stability Studies

Transdermal films loaded with an extract of *Benincasa hispida* (Thunb.) Cogn. were subjected to stability testing to ascertain their physical stability and the potency of the medicine contained within.

Table 8: Stability studies of F3 formulation

Sampling Intervals in days	Drug content 25°C/60% HR	Drug Content 30°C/65% HR	Drug content 40°C/75% HR
15	98.44	98.26	98.16
45	98.32	98.20	98.08
60	98.19	98.10	98.08

Diffusion Study

All formulations were subjected to diffusion testing to determine how well their in vitro extracts dispersed.

Table 9: In vitro Diffusion profile of Benincasa hispida (Thunb.) Cogn. extract loaded Formulation F1 (2.5 mg/cm^2)

Times (hrs)	√T	LogT	%Cumulative extract released	Log %Cum extract released	%Cum extract Retained	Log %Cum extract Retained
0	0	0	0	0	0	0
1	1.000	0.000	13.72	1.134	86.28	1.934
2	1.123	0.083	16.93	1.228	83.08	1.916
3	1.632	0.212	22.32	1.344	77.63	1.890
4	1.900	0.280	28.82	1.458	72.10	1.568
5	2.147	0.370	35.64	1.558	65.10	1.808
6	2.800	0.421	43.00	1.638	57.12	1.758
7	3.400	0.484	48.90	1.700	52.02	1.708

Table 10: *In vitro* diffusion profile of *Benincasa hispida* (Thunb.) Cogn. extract loaded Formulation F2(2.5 mg/cm^2)

Time (hrs)	√T	Log T	% Cumulative extract Released	Log %Cum extract Released	% Cumulative extract Retained	Log %Cum extract Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.32	1.154	85.64	1.936
2	1.212	0.082	20.20	1.303	79.76	1.899
3	1.630	0.210	24.46	1.386	75.70	1.876
4	1.898	0.276	32.32	1.507	67.64	1.828
6	2.147	0.368	37.80	1.575	62.16	1.771
8	2.726	0. 432	40.60	1.667	59.36	1.768
10	3.060	0.483	51.18	1.706	49.78	1.677
12	3.362	0.516	58.22	1.718	47.74	1.618

Table 11: *In vitro* diffusion profile of *Benincasa hispida* (Thunb.) Cogn. extract loaded Formulation F3 (2.5 mg/cm^2)

Time (hrs)	√T	Log T	% Cumulative extract Released	Log %Cum extract Released	% Cumulative extract Retained	Log %Cum extract Retained
0	0	0	0	0	0	0
1	1.000	0.000	15.20	1.180	84.76	1.926
2	1.212	0.082	22.32	1.347	77.64	1.888
3	1.630	0.210	30.12	1.477	69.84	1.842
4	1.898	0.276	40.80	1.609	59.16	1.770
6	2.147	0.368	47.92	1.679	52.04	1.715
8	2.726	0.432	53.10	1.723	46.86	1.669
10	3.060	0.483	63.70	1.802	36.26	1.558
12	3.362	0.516	70.26	1.845	29.70	1.471

Table 12: *In vitro* diffusion profile of *Benincasa hispida* (Thunb.) Cogn. extract loaded Formulation F4 (2.5mg/cm^2)

Time (hrs)	√T	Log T	% Cumulative extract Released	Log %Cum extract Released	% Cumulative extract Retained	Log %Cum extract Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.26	1.153	85.70	1.931
2	1.212	0.082	20.84	1.317	79.12	1.896
3	1.630	0.210	28.72	1.456	71.24	1.851
4	1.898	0.276	38.46	1.583	61.50	1.787
6	2.147	0.368	40.92	1.610	59.04	1.769
8	2.726	0. 432	41.78	1.619	58.18	1.762
10	3.060	0.483	42.68	1.628	57.18	1.756

12	3.362	0.516	50.64	1.702	49.32	1.691

Table 13: *In vitro* diffusion profile of *Benincasa hispida* (Thunb.) Cogn. extract loaded Formulation F5 (2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative extract Released	Log %Cum extract Released	% Cumulative extract Retained	Log %Cum extract Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.84	1.170	85.12	1.928
2	1.212	0.082	21.70	1.334	78.26	1.892
3	1.630	0.210	29.82	1.476	70.14	1.844
4	1.898	0.276	40.10	1.601	59.86	1.775
6	2.147	0.368	42.14	1.622	57.14	1.750
8	2.726	0.432	50.82	1.701	49.14	1.689
10	3.060	0.483	52.84	1.721	47.12	1.671
12	3.362	0.516	56.86	1.752	43.10	1.632

Acute toxicity results

Extracts at doses up to the maximum recommended by OECD guideline 423 (2000 mg/kg p.o.) were given to a group of animals, and there was no evidence of toxicity. It was found that there were no symptoms or negative side effects. Therefore, the maximum tolerated dosage was utilized as the therapeutic dose for subsequent pharmacological research. Based on the results of this study, we conclude that 200mg/kg and 400mg/kg of *Benincasa hispida* (Thunb.) Cogn. extracts are the lowest and maximum therapeutic dosage levels, respectively.

In vivo results

Paw edema volume

Table 14: Effect of *Benincasa hispida* (Thunb.) Cogn. extracts on changes in paw volume in CFA induced arthritis in rats

Group	0th day	9th day	18th day	25th day	40th day
Control	0.62 ± 0.01	0.64 ± 0.01	0.63 ± 0.02	0.65 ± 0.01	0.65 ± 0.02
Negativecontrol	0.64 ± 0.02	0.73 ± 0.01	0.78 ± 0.01	0.84 ± 0.02	0.89 ± 0.02
Standard	0.57 ± 0.01	0.46 ± 0.01	0.41 ± 0.02	0.38 ± 0.01	0.33 ± 0.02
200 mg/kgBH	0.60 ± 0.02	0.49 ± 0.01	0.40 ± 0.02	0.38 ± 0.01	0.36 ± 0.01*
400 mg/kgBH	0.56 ± 0.02	0.40 ± 0.01	0.34 ±0.01*	0.30 ±0.01**	0.25 ± 0.01*

Values are expressed in mean ± SEM, n = 6, *p<0.05 are significant compared to standard, LDBH (200 mg/kg); HDBH (400 mg/kg)

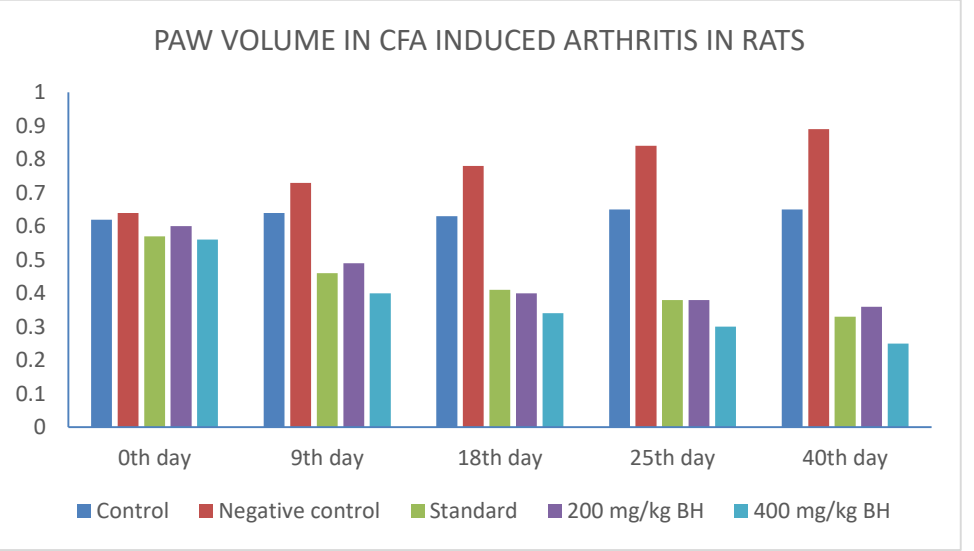

Figure 2: Effect of *Benincasa hispida* (Thunb.) Cogn. extracts on changes in paw volume in CFA induced arthritis in rats

Locomotor activity and body weight

Over the course of the trial, the rats' body weights fluctuated in response to the increasing prevalence and severity of arthritis. Weight loss during arthritis was also supported by prior data on metabolic changes in ill rats. Within the control group, participants' weights stayed rather stable throughout the course of the study's 40 days. After 9 days of treatment, both the low-dose (100 mg/kg) and high-dose (200 mg/kg, 400 mg/kg) groups of rats saw a decrease in body weight, with weight loss being statistically significant on days 18 and 25. No discernible weight loss or gain occurred as a result of methotrexate therapy. Non-treated rats did not exhibit any noticeable behavioral changes. On days 9 and 18, both the plant extract and the control group showed significantly reduced rat activity. By day 40 of the trial, however, normal mobility had returned, especially as compared to the untreated groups.

Table 14: Effect of ethanolic extracts of LDBH and HDBH on CFA induced arthritic rats showing changes in body weight and locomotor activity

Group	Physical and behavioural changes									
	0^{th} day		9^{th} day		18^{th} day		25^{th} day		40^{th} day	
	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)	BW (gms)	M (sec)
Control	178 ±0.02	20	173 ±0.01	22	168 ±0.01	24	171 ±0.02	22	172±0.01	23
Negative control	168 ±0.01	20	158 ±0.01	30	148 ±0.02	35	98 ±0.02	50	90 ±0.02	55
Standard	168 ±0.01	20	158 ±0.02	30	138 ±0.01	32	128 ±0.01	32	173 ±0.01	25
LDBH	158	20	148	30	138	30	118	40	98	40

	±0.02		±0.02		±0.02		±0.01		±0.01	
HDBH	199 ±0.02	20	178 ±0.02	25	158 ±0.02	25	163 ±0.01	30	193 ±0.01*	25

Values are expressed in mean ± SEM, n=6, *p<0.05 are considered significant compared to standard. LDBH (200 mg/kg); HDBH (400 mg/kg); BW - Body weight; M – Movement.

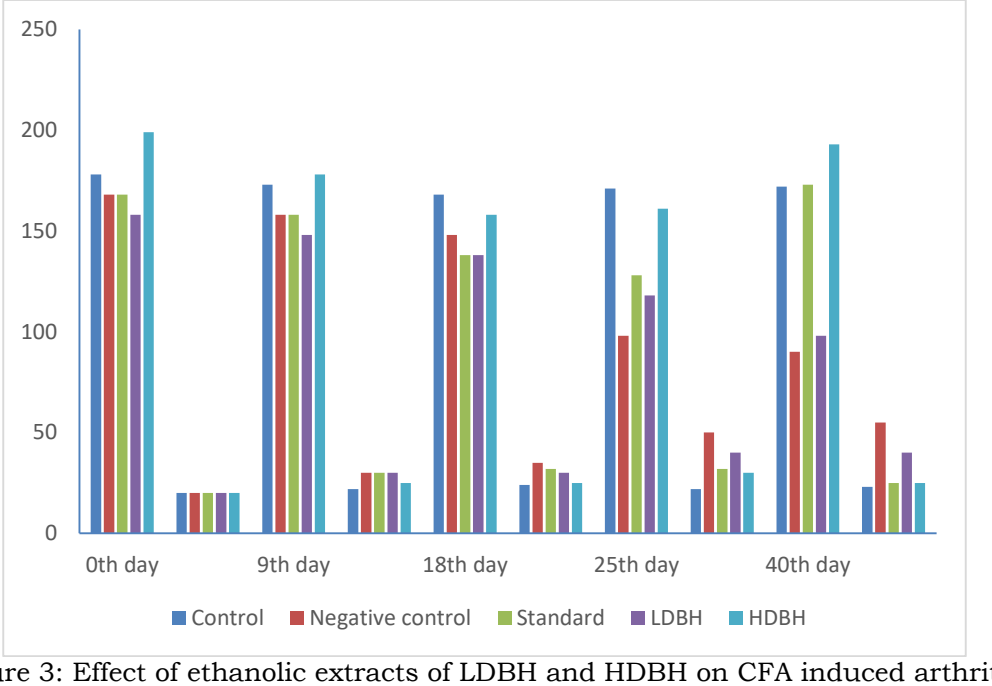

Figure 3: Effect of ethanolic extracts of LDBH and HDBH on CFA induced arthritic rats showing changes in body weight and locomotor activity"

Conclusion

The films can be made with a constant drug content and little batch variability using the method used. Spectrophotometric analysis at 260 nm was used to determine drug concentration. Drug concentration ranges from 2.37 mg/cm^2 to 2.48 mg/cm^2 across all formulations, suggesting reasonable consistency. The surface morphology of the film was analyzed by scanning electron microscopy, and the results validated the film's smooth, glossy, and aesthetically attractive look. An indicator of Benincasa hispida's anti-arthritic activity at doses of 200&400 mg/kg, po, is paw swelling. Increasing SA content resulted in a larger film viscosity (measuring between 392.13 and 403.45 Cp).

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