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FORMULATION AND EVALUATION OF NIGELLA SATIVA TRANSDERMAL PATCHES FOR ANTI-CANCER ACTIVITY

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ABSTRACT

The seeds of Nigella sativa L., commonly known as black cumin seeds, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The pharmacological studies have shown that the aqueous and oil extracts of the seeds have been shown to possess antioxidant, anti-inflammatory, anticancer, analgesic and antimicrobial activities. Thymoquinone, the most abundant constituent of black seed essential oil, has been shown to be the active principle responsible for many of the seeds' beneficial effects. In this study, Transdermal Patches containing Nigella sativa (NS) were prepared by Solvent Casting Technique using two different natural polymers, Pectin and Bovine Serum Albumin in various ratios using Polyethylene Glycol 400 as enhancer. The prepared patches were studied and optimized with respect to physicochemical characters, drug-excipient interaction, in-vitro dissolution, release kinetics studies. The combination of Pectin and Polyethylene Glycol produces smooth flexible films. The In-vitro Dissolution studies revealed that the cumulative amount of drug released was decreased as the polymer content of the film increased. Based on the in-vitro drug release studies, P2 [NS-Pectin (1:0.5)] was found to be better formulation with reliable physicochemical characteristics and it released a maximum amount of drug and followed zero order kinetics. It is concluded as the optimized formulation for effective drug delivery and can be targeted to the cancer cells and produce sustained drug delivery.

KEYWORDS: Transdermal patches, Nigella sativa, Pectin, Bovine Serum Albumin, Solvent Casting Technique, Franz Diffusion Cell, Anticancer activity.

INTRODUCTION

Novel drug delivery system (NDDS) is a novel approach to drug delivery that addresses the limitations of the traditional drug delivery systems which is advantageous in giving up the drug at predetermined rate by targeting just the affected zone inside a patient's body and delivering the drug only at the site of action which helps in minimizing the toxic effects with an increase in bioavailability of drugs. In general, our country has a vast knowledge base on Herbal medicines whose potential is only being realized in recent years. However, the drug delivery system used for administering the herbal medicine to the patient is traditional and out-of-date, resulting in reduced efficacy of the drug. If the novel drug delivery technology is applied in herbal medicine, it may help in increasing the efficacy and reducing the side effects of various herbal compounds and herbs. This is the basic idea behind incorporating novel method of drug delivery in herbal medicines. For a long time, herbal medicines were not considered for development as novel formulations owing to lack of scientific justification and processing difficulties, such as standardization, extraction and identification of individual drug components in complex polyherbal systems. However, modern phytopharmaceutical research can solve the scientific needs (such as determination of pharmacokinetics, mechanism of action, site of action, accurate dose required, etc.) of herbal medicines to be incorporated in NDDS, such as Nanoparticles, Solid Lipid Nanoparticles, Microspheres, Magnetic Microspheres, Microemulsions, Solid Dispersions, Liposomes, Niosomes, Proniosomes, Transdermal Patches, Matrix systems, etc.[1]

Transdermal Patch: Transdermal drug delivery system (TDDS patch) are self-contained discrete dosage forms, when applied to the intact skin, are designed to deliver the drug through the skin at a controlled rate to the systemic circulation.[2] Since the herbal TDDS utilizes the skin as a site for continuous drug administration, this delivery system helps in improving the absolute bioavailability of the drug by avoiding hepatic first-pass metabolism of the drug in liver and gastrointestinal tract and moreover, this scheme offers a prolonged drug delivery with infrequent dosing via zero-order kinetics and also this therapy can be easily fired at any time.[3,4]

Nigella sativa: Nigella sativa (NS) is an annual herbaceous (non woody) flowering plant (angiosperm) that originated in the Mediterranean region but has been cultivated in other parts of the world such as Asia, Africa and the Arabian Peninsula and is known for its remarkable healing powers. It has been used for centuries all over the world to treat many

ailments such as headaches, nasal congestion, toothache, intestinal worms, asthma, diarrhea and dyslipidaemia, a good remedy for poisonous bites and stings and much more. An amazing discovery was made in South Carolina at the Cancer Research Laboratory of Hilton Head Island, where experimental research proved that N.sativa oil had a success on tumor therapy without causing the negative side effects caused by chemo therapy. It was actually found that N.sativa increased the growth rate of bone marrow by approximately 250%. Not only that but it was also discovered that N. sativa suppresses tumor growth by 50%.[5,6,7]

Many of the anti-cancer activities of N. sativa have been attributed to its major active constituent, thymoquinone (TQ). TQ has been shown to exert anti-proliferative, pro-apoptotic, anti-oxidant, anti-mutagenic, anti-angiogenic, and anti-metastatic effects against cancer cells. TQ seems to mediate its anti-cancer effects by targeting a number of cellular pathways involving p53, NF-κB, PPARγ, STAT3, MAPK, and PI3K/AKT transducing signals. Besides TQ, α-hederin, a pentacyclic triterpene saponin found in N. sativa seeds also exerts effective anti-cancer effects, both in-vitro and in-vivo. Moreover, other phytoconstituents like thymohydroquinone, thymol, carvacrol, nigellicine, nigellidine, have been demonstrated to play anti-cancer and cytotoxic functions against cancer cells.[8,9]

MATERIALS AND METHODS

MATERIALS

Nigella sativa seeds – Herbal Plant and Powder Shop, Coimbatore, Tamil Nadu, India, **Pectin, Bovine Serum Albumin** – Loba, Mumbai, **Polyethylene Glycol 400, Methanol** – Hi-Pure Fine Chem Industries, Chennai, **Glycerine** – Merck Ltd., Mumbai, **Soxhlet** – Remi Equipments, Pvt. Ltd., **Rotary Evaporator** – Superfit Pvt. Ltd., **FTIR** – Perkin-Elmer, USA, **UV Spectrophotometer** – Shimadzu 1700, Japan.

METHODOLOGY

***Extraction of seeds of Nigella sativa*[6,10]**

Seeds of Nigella sativa were purchased from the Herbal Plant and Powder Shop in Coimbatore, Tamil Nadu, India and authenticated by Botanical Survey of India (BSI), Coimbatore. The seeds were cleaned, shade dried at room temperature and coarsely grounded using a table top mixture. About 200g of the dried powder was extracted continuously using methanol in Soxhlet Apparatus for 3hrs and stored in an amber glass screw cap bottle at room temperature until use. Then the methanolic extract was subjected to evaporation by Rota

Evaporator for about 3hrs at 30°C to obtain the crude extract (7.4%w/v). The extract was dried under vacuum in oven.

Preparation of Calibration curve of Nigella sativa Extract[11]

Accurately weighed quantity (100mg) of Nigella sativa was transferred into a 100ml volumetric flask and dissolved in small amount of methanol and made up to the volume to make the standard stock solution of 1 mg/ml. From the stock, 1ml was taken in 10ml volumetric flask and made up the volume with the buffer. From this solution make a dilution of 1 µg/ml, 2µg/ml, 4 µg/ml, 6 µg/ml, 10µg/ml, 14µg/ml, and 16 µg/ml. The absorbance of these solutions was determined at 254nm using UV spectrophotometer. The calibration curve was constructed between the absorbance and concentration.

Formulation of a Transdermal Patch[12,13]

Transdermal patches of all batches were prepared by Solvent Casting Method using methanolic extract of N.sativa with two different natural polymers in six different ratios (1:0.25, 1:0.5, 1:0.75, 1:1, 1:1.25 & 1:1.5). Weighed quantity of polymer was dissolved in calculated quantity of water and heated on a water bath. Calculated amount of Nigella sativa extract was added to the above mixture and stirred well until a homogenous mixture was formed. Then calculated amount of PEG 400 was added as a plasticizer. Glycerin was added as a permeation enhancer. The polymeric drug solution was allowed to stand for 15 min to remove air bubbles and poured into a petridish and air dried at room temperature in a dust free environment for 24hrs. An inverted funnel was covered over the petridish to avoid evaporation of the solvent. The patches were then peeled off from the petridish with the help of a knife and packed in an aluminium foil and kept in desiccator.

Evaluation of Formulations[14,15]

1. *Uniformity of weight*

This was done by weighing five different patches of individual batch taking uniform size at random and calculating the average weight of three. The tests were performed on patch which was dried at 60˚C for 4hrs prior to testing.

2. *Thickness of the patch*

The Thickness of the patch was assessed by using digital vernier caliper at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined.

3. *Drug content determination*

The patches were taken and added to a beaker containing 100 ml of distilled water. The medium was stirred with a magnetic bead for 5 hrs. The solution was later filtered and analyzed for drug content with proper dilution at 254 nm spectrophotometrically.

4. *Folding Endurance*

This was determined by repeatedly folding one patch at the same place till it broke. The number of times the patch could be folded at the same place without breaking gave the value of folding endurance.

5. *Flatness study*

This study was conducted to appraise that the prepared transdermal patches possess a smooth surface and shall not constrict with time. Longitudinal strips were cut-out from each film, one from the center and two from either side. The length of each strip was measured, and then the variation in the length due to the non-uniformity in flatness was measured. Flatness calculated by measuring percentage constriction of strips and a 0% constriction was considered to be equal to a 100% flatness. It is calculated by

$$\% \text{ constriction} = (l_1 - l_2) / l_1 \times 100$$

Where, l_1 = Initial length of each strip; l_2 = Final length of each strip.

6. *Percentage moisture uptake*

The patch were weighed accurately and placed in desiccators containing activated aluminium silica at room temperature. After 24hrs, individual films were weighed repeatedly until they showed a constant weight. The percentage moisture uptake was calculated as the difference between final and initial weight with respect to initial weight. It is calculated by using following formula.

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{ X } 100$$

7. *Percentage Moisture Content*

The patch were weighed and kept in desiccators at room temperature. After 24hrs the patch were taken out and exposed to 84% relative humidity (a saturated solution of sodium chloride) in desiccators until a constant weight for the film was obtained. The percentage moisture content was calculated using the following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ X } 100$$

8. *Determination of surface pH*

The patches were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 hrs at room temperature and pH was noted by bringing the electrode in contact with the surface of the patch, allowing it to equilibrate for 1 min.

9. *Percent Elongation*

When stress is applied, a patch sample stretches and this is referred to as strain. Strain is basically the deformation of patch divided by original dimension of the sample. Generally elongation of patch increases as the plasticizer content increases. It is calculated by using following formula.

$$\text{Percentage Elongation} = \frac{\text{Increase in length of patch}}{\text{Initial length of patch}} \text{ X } 100$$

10. *Tensile Strength*

Tensile Strength is the maximum stress applied to a point at which the patch specimen breaks.

It is calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below

$$\text{Percentage Elongation} = \frac{\text{Load at failure}}{\text{Patch Thickness X Patch width}} \text{ X } 100$$

11. *In-vitro Drug Release Studies* [16]

Franz Diffusion Cell was employed for the in-vitro characterization of transdermal formulations. This is a reliable method for the prediction of drug transport across the skin from topical formulations. The receptor compartment of the diffusion cell was filled with 30.0 ml of phosphate buffered saline (pH 7.4), and in-vitro drug release studies were carried out using synthetic cellophane membrane. The prepared formulations were applied onto the

cellophane membrane. The assembly was constantly maintained at 37.0 ± 2.0 °C at 50 rpm. Samples (1.0 ml aliquots) were then withdrawn at suitable time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 18 and 24hrs) and replaced with 1ml of medium to maintain the receptor phase volume to 30 ml. The samples were analyzed spectrophotometrically at 254 nm.

RESULTS AND DISCUSSION

Physicochemical Evaluation

The two batches P (P1, P2, P3, P4, P5, P6) and B (B1, B2, B3, B4,B5, B6) of extract loaded patches with different ratios of two different natural polymers (Pectin and Bovine Serum Albumin) were subjected to various physicochemical evaluations and the data profile are given in Table 2. Based on thickness, uniformity of weight, folding endurance, percentage flatness, surface pH, percentage moisture uptake & moisture content, the formulations **P2** and **B4** were selected for further studies.

In-vitro Drug Release Studies

In-vitro drug release study of the prepared Nigella sativa Transdermal patches was carried out using dialysis Franz Diffusion Cell. Amount of drug released in different time intervals were observed. In-vitro drug release profile data of Nigella sativa Transdermal patches containing Pectin (P1- P6) are given in Table 3 and Figure 3 where Bovine Serum Albumin (B1- B6) are given in Table 4 and Figure 4. From this, the formulation **P2** has a release of **85.52%** at **24hrs**. Thereby, in accordance with the Physicochemical Evaluation and In-vitro Release studies, the formulation **P2** may be concluded as optimized formulation for effective drug delivery.

Stability Studies

The prepared films were wrapped in aluminum foil. The aluminum foils were placed in a stability chamber whose temperature was maintained at 40°C ± 2°C at 75% RH for 3 months as per ICH guidelines. Then, films were withdrawn and evaluated for physical parameters like colour, thickness and weight of the films. During this period the formulation were stable and showed no significant changes in visual appearance, colour, texture and drug content.

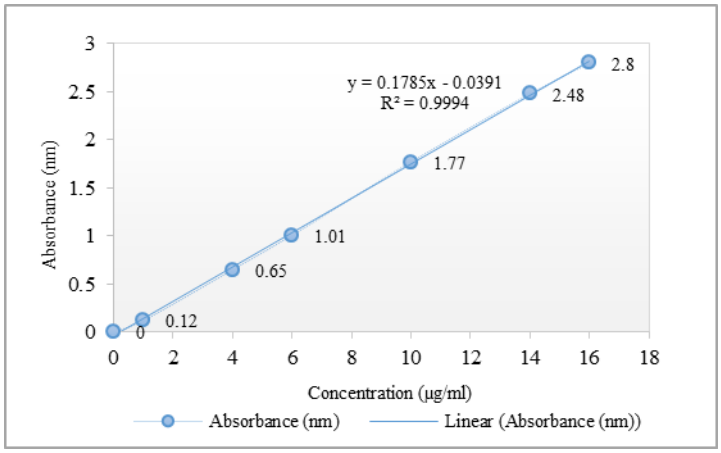

Fig. 1: ***Calibration Curve of Nigella sativa Extract in UV Spectroscopy.***

Table 1: ***Optimized Formula of Nigella sativa Transdermal patches.***

S.No.	Formulation code	Drug	Polymer (in g)	Drug: Polymer ratio
1.	P1	Methanolic extract of NS 2 ml (1.58gm)	Pectin (0.39)	1:0.25
2.	P2		Pectin (0.79)	1:0.5
3.	P3		Pectin (1.18)	1:0.75
4.	P4		Pectin (1.58)	1:1
5.	P5		Pectin (1.97)	1:1.25
6.	P6		Pectin (2.37)	1:1.5
7.	B1		BSA (0.39)	1:0.25
8.	B2		BSA (0.79)	1:0.5
9.	B3		BSA (1.18)	1:0.75
10.	B4		BSA (1.58)	1:1
11.	B5		BSA (1.97)	1:1.25
12.	B6		BSA (2.37)	1:1.5

NS – Nigella sativa; **BSA** – Bovine Serum Albumin

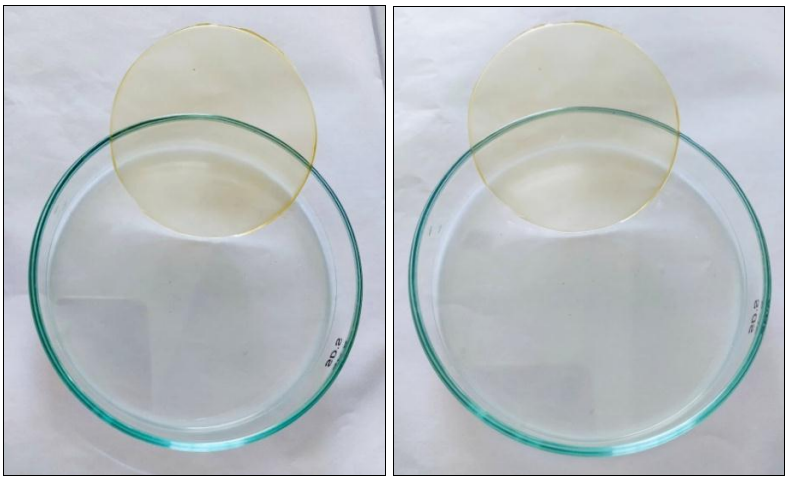

Fig. 2: ***Preparation of Nigella sativa Transdermal Patches.***

Table 2: ***Physicochemical characterization of Nigella sativa Transdermal patches.***

Formulation code	Uniformity of weight (g)	Thickness (mm)	Drug content (%)	Folding Endurance (no's)	Moisture Uptake (%)	Moisture Content (%)	Flatness	Surface pH
P1	0.287	0.10	90.99	17	4.91	2.50	100	7.1
P2	**0.299**	**0.10**	**93.21**	**19**	**4.93**	**3.10**	**100**	**7.4**
P3	0.301	0.12	91.23	17	5.02	2.61	100	7.3
P4	0.300	0.15	92.69	16	5.14	2.72	100	7.2
P5	0.306	0.18	90.98	16	5.79	2.95	100	7.3
P6	0.290	0.21	89.25	15	5.85	3.07	100	7.0
B1	0.38	0.12	81.05	12	4.72	2.59	100	7.1
B2	0.39	0.15	81.78	12	4.84	3.02	100	7.0
B3	0.39	0.13	82.03	14	4.91	3.15	100	7.1
B4	**0.41**	**0.14**	**82.54**	**16**	**5.03**	**3.72**	**100**	**7.3**
B5	0.48	0.18	83.35	16	5.59	3.95	100	7.2
B6	0.49	0.23	81.25	15	5.78	3.91	100	7.3

Table 3: ***In-vitro release data profile of Nigella sativa Transdermal patches (P1- P6).***

S.No.	Time (hrs)	Cumulative percentage drug release (%)					
		P1	P2	P3	P4	P5	P6
1.	0	0	**0**	0	0	0	0
2.	1	6.78	**4.2**	3.6	2.85	2.53	1.87
3.	2	12.31	**10.6**	8.56	5.83	4.78	3.95
4.	3	15.65	**17.21**	12.89	9.65	5.64	5.23
5.	4	25.89	**24.96**	18.56	11.26	8.15	6.98
6.	5	31.1	**30.54**	26.45	20.38	12.45	8.53
7.	6	35.54	**34.34**	31.53	25.46	19.82	12.32
8.	7	40.68	**38.75**	35.46	30.25	22.38	18.03
9.	8	45.7	**43.23**	42.41	34.5	28.48	23.54
10	10	51.77	**49.58**	47.23	40.26	38.73	40.01
11.	12	62.1	**55.25**	52.36	50.89	49.24	48.43
12.	18	78.38	**73.46**	70.86	69.87	67.28	61.24
13.	24	91.24	**85.52**	83.21	79.52	80.25	70.32

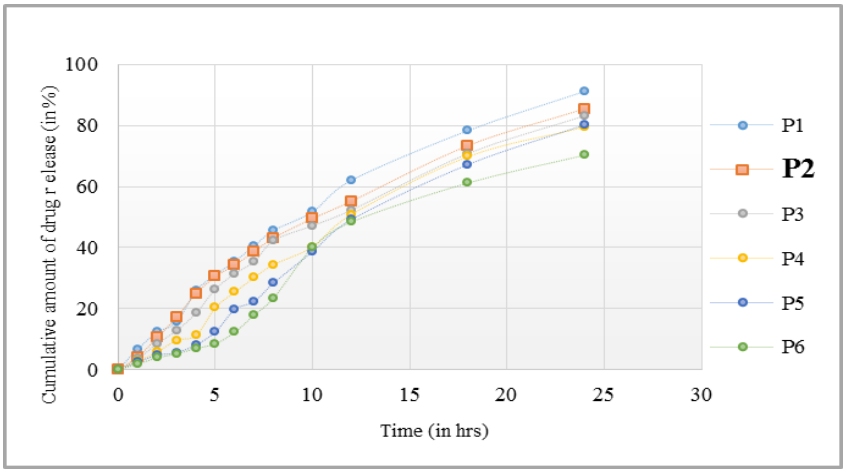

Fig 3: Comparison of in-vitro release profiles of formulations (P1- P6).

Table 4: ***In-vitro release data profile of Nigella sativa Transdermal patches (B1- B6).***

S.No.	Time (hrs)	Cumulative percentage drug release (%)					
		B1	B2	B3	B4	B5	B6
1.	0	0	0	0	**0**	0	0
2.	1	10.92	8.14	7.56	**5.48**	3.89	3.98
3.	2	13.95	11.04	10.23	**8.98**	5.87	4.78
4.	3	17.86	14.42	13.26	**11.21**	10.57	5.64
5.	4	22.89	19.86	16.89	**14.96**	13.49	7.15
6.	5	30.98	25.24	21.96	**20.54**	15.21	11.45
7.	6	41.04	32.99	28.54	**25.34**	20.48	14.82
8.	7	47.68	42.52	32.95	**29.45**	24.91	19.38
9.	8	55.25	47.01	38.97	**34.23**	35.48	25.48
10	10	64.67	57.65	44.52	**49.58**	44.25	37.73
11.	12	70.21	67.63	52.46	**57.25**	53.87	49.24
12.	18	79.05	80.71	78.32	**72.89**	75.56	67.28
13.	24	95.68	94.84	92.45	**89.87**	90.58	91.22

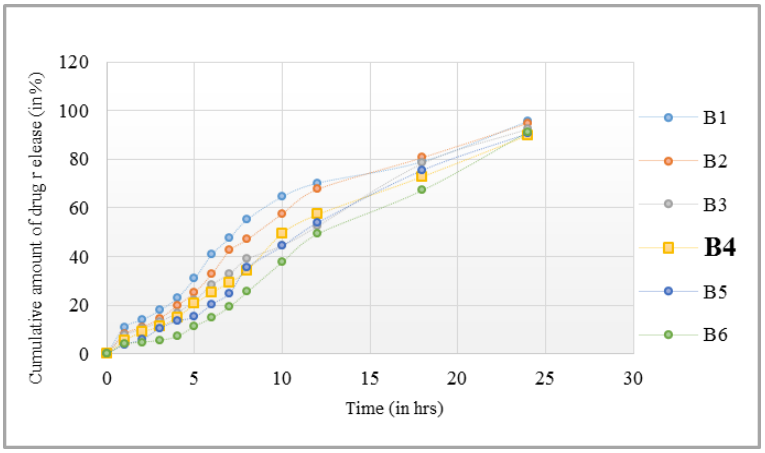

Fig 4: ***Comparison of in-vitro release profiles of formulations (B1- B6).***

Table 5: ***In-vitro Drug Release kinetics values of P2.***

Time (in hrs)	Sq.root of Time	Log Time	Cumulative % Drug release	Log Cumulative % Drug release
0	0	-	0	-
1	1	0	4.20	0.6232
2	1.414	0.3010	10.60	1.0253
3	1.732	0.4771	17.21	1.2357
4	2	0.6020	24.96	1.3972
5	2.236	0.6989	30.54	1.4848
6	2.449	0.7781	34.34	1.5358
7	2.645	0.8450	38.75	1.5882
8	2.828	0.9030	43.23	1.6357
10	3.162	1.000	49.58	1.6953
12	3.464	1.0791	55.25	1.7423
18	4.242	1.2552	73.46	1.8660
24	4.898	1.3802	85.52	1.9320

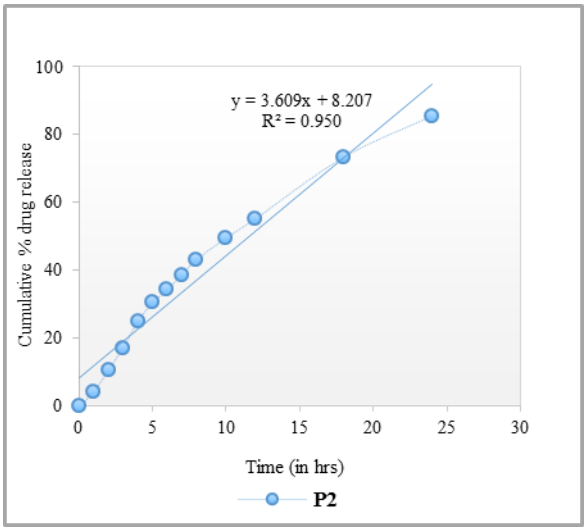

Fig 5: Zero order Kinetic Plot of P2.

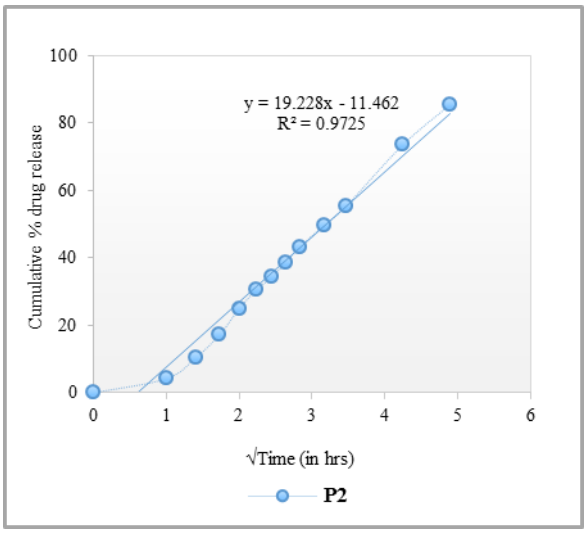

Fig 6: Higuchi Plot of P2

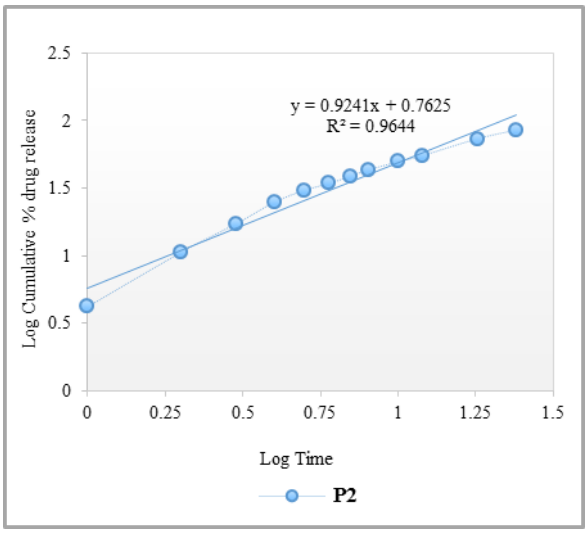

Fig 7: Korsmeyer Peppas Plot of P2

Table 6: ***Kinetic release studies of P2.***

Formulation code	Correlation Coefficient (R^2)		
	Zero order	***Higuchi***	***Korsmeyer peppas***
P2	0.950	0.972	0.964

SUMMARY AND CONCLUSION

It can be concluded that herbal drugs in the form of extracts can also be used in formulating transdermal patches by Solvent Casting method as a novel approach. The study showed that, the plasticizer, Polyethyleneglycol 400 diffuses through the patch and softens the polymer particles. This softening promotes latex coalescence and patch formation and was suitable for good flexibility and elasticity. Based on the physicochemical parameters and in-vitro release studies, it was found that the formulation **P2** containing Nigella sativa and Pectin (1:0.5),

considered as optimized and showed **85.52%** release **at the end of 24 hours** and also it showed satisfactory results in the evaluation tests. Also it follows zero order kinetics and Higuchi's and Korsmeyer - Peppas model as release mechanism and emerge as an excellent sustained drug delivery system and can be targeted for the effective treatment of different types of cancer cells.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Jain N, Valli K, Devi V. Importance of novel drug delivery systems in herbal medicines. Pharmacognosy Reviews, 2010; 4(7): 27.
2. Hardainiyan S, Chandra Nandy B, Jasuja N, Vyas P, K Raghav P. A Review on the recent innovations in transdermal drug delivery for herbal therapy. Journal of Biomedical and Pharmaceutical Research, 2014; 3(3): 88-101.
3. Tanwar H, Sachdeva R. Transdermal Drug Delivery System: A Review. International Journal of Pharmaceutical Sciences and Research, 2016; 7(6): 2274-2290.
4. Patel D, Chaudhary SA, Parmar B, Bhura N. Transdermal drug delivery system: a review. The pharma innovation, Jun 1, 2012; 1(4): 66-75.
5. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific journal of tropical biomedicine, May 1, 2013; 3(5): 337-352.
6. Sudhir SP, Deshmukh P, Verma HN. Comparative study of antimicrobial effect of Nigella sativa seed extracts from different geographies. Int. J. Pharmacogn, 2016; 3: 257-264.
7. Almatrafi A A. Medicinal Uses of Nigella Sativa (Black Seeds). International Journal of Alternative Medicine, 2016; 21(1): 1129-1131.
8. Majdalawieh AF, Fayyad MW. Recent advances on the anti-cancer properties of Nigella sativa, a widely used food additive. Journal of Ayurveda and integrative medicine, Jul 1, 2016; 7(3): 173-180.

9. Banerjee S, Padhye S, Azmi A, Wang Z, Philip PA, Kucuk O, Sarkar FH, Mohammad RM. Review on molecular and therapeutic potential of thymoquinone in cancer. Nutrition and cancer, Sep 23, 2010; 62(7): 938-946.
10. Zuridah H, Fairuz AR, Zakri AH, Rahim MN. In vitro antibacterial activity of Nigella sativa against Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and Bacillus cereus. Asian journal of plant sciences, 2008; 7(3): 331–333.
11. Taleuzzaman M, Gilani S. Analytical Profile of Anti-Diabetic Constituents–Thymoquinone from Seed of Nigella sativa. Int. J. ChemTech Res., 2017; 10: 139-147.
12. Sakhare Abhaykumar D, Biyani Kailash R, Sudke Suresh G. Design and optimization of reservoir type transdermal patches of carvedilol. Skin, 2019; 8(6): 149-157.
13. Setty M M, Swarnkar S, Laxane N, Laxane S. Design, Development and Evaluation of Herbal Transdermal Patches for Anti-Inflammatory Activity. International Journal of Pharmacy, 2018; 8(3): 33-50.
14. Dhanalakshmi S, Harikrishnan N, Devi M, Keerthana V, Vijayalakshmi. Fabrication and evaluation of herbal transdermal flim from Hibiscus Rosa sinensis. Int J Curr Pharm Res., 2019; 11(5): 101-105.
15. Mutalik S, Udupa N. Formulation development, in vitro and in vivo evaluation of membrane controlled transdermal systems of glibenclamide. J Pharm Sci., Jan 21; 2005; 8(1): 26-38.
16. Alotaibi FO, Mustafa G, Ahuja A. Study of enhanced anti-inflammatory potential of nigella sativa in topical nanoformulation. Int. J. Pharm. Pharm. Sci., 2018; 10(7): 41-51.