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Formualtion and evalaution of transdermal patch for the treatment of inflammation

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Abstract

Background: Quercetin is one of the important bioflavonoids present in more than twenty plants material and which is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities. Free-radical are one of the key factors for the development of the diseases such as hypertension, vascular disorders, and metabolic syndrome. The objective of this study was to develop a transdermal drug delivery system for Quercetin as a once daily dosage form.

Methods: Transdermal patches were prepared by solvent casting technique employing controlled release grades of HPMC and ethyl cellulose in presence of plasticizer PEG. Standard procedures were used to analyze the prepared films for various physicochemical parameters, drug release (Franz diffusion cell) and skin irritation test.

Results: The formulations were uniform in their physical characteristics with low water vapor absorption, uniformity in patch characteristics. The patches were devoid of hypersensitivity reactions on rat skin. The in vitro release of formulation Q1, Q2, Q3, Q4, Q5 & Q6 has shown release of about 57.02%, 52.66%, 85.77%, 74.78%, 64.27%, and 48.08%at 24 h and respectively. The order of drug release was found to be Q3>Q4>Q5>Q1>Q2>Q6. Anti-inflammatory activity by Carrageenan induced Paw edema model formulation code Q3 reduced the paw edema in 4th hour to 0.24±0.020 which was found to be highly significant when compared to control 0.69±0.069 and standard Nu Patch 200 mg i.e 0.20±0.024. In Xylene induced mouse ear edema model formulation code Q3 showed 31.16 (% edema) which was found significant to when compared to controlled 88.15 (% edema)

Key words- Patch, Quercetine, Flavonoids, Transdermal, Inflammation

1.0 INTRODUCTION

Transdermal drug delivery systems (TDDSs) can be defined as self-contained discrete dosage forms which, when applied to the intact skin, deliver the drug(s) through the skin portal at a predetermined and reproducible rate into the systemic circulation over a prolonged period of time **(Prabhakar et al. 2013; Prausnitz et al. 2004; Gupta et al. 2009).**

The goal of dosage design for transdermal products is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin. Transdermal delivery provides a leading edge over injectable and oral routes by increasing patient compliance and avoiding first pass metabolism, respectively **(Selvam et al. 2010).**

The market share for transdermal delivery was $12.7 billion in the year 2005, which rose to $21.5 billion in the year 2010 and is expected to increase to $31.5 billion in the year 2015. In the recent past, several innovative technologies have come up in an attempt to enhance transdermal drug delivery for therapeutic and diagnostic purposes for targeting the delivery of the drugs to specific tissues **(Jain and Vyas 1994; Ilana and Joseph 2004; Barry 1987).** The formulation of drugs into a transdermal drug de- livery system requires a selection of physicochemical and biological properties **(Rani et al. 2011; Izumoto et al. 1992)**.

2.0 MATERIALS AND METHODS

2.1 Selection of Drug: Quercetin is one of the most abundant natural flavonoid and was selected for the preparation of a transdermal patch.

2.2 Preformulation Studies of Drug

It includes Identification, Melting point, calibration curve**,** Fourier Transform Infra-Red analysis, Solubility Studies, Partition coefficient**,** thin layer chromatography and Drug-Excipient Interaction.

2.3 Identification of Drug

a) Organoleptic properties

Organoleptic characteristics of the drug were investigated on the basis of color, odor, taste and State.

b) Melting Point: Melting point determination of quercetin was done by using Melting Point Apparatus.

c) UV Absorption Maxima: The identification of drug was done by UV spectrophotometric method. From the spectra, λmax of quercetin was observed at 256 nm. The spectral data from this scan was used for the preparation of calibration curve of quercetin **(Xiao *et al*, 2006).**

d) Fourier Transform Infra-Red analysis: The FTIR analysis of the sample was carried out for compound identification (FTIR-8400S Shimadzu). The powdered drug was placed carefully over sample holder ensuring no air entrapment, thereafter the sample was scanned.

e) **Solubility:** The solubility analysis for Quercetin was done in different solvents like Ethanol, Methanol, Water, PBS (pH 7.2), Methanol: PBS pH 7.4 (05: 95), Methanol: PBS pH 7.4 (10 : 90), Methanol : PBS pH 7.4 (20 : 80).

f) Partition Coefficient: Partition coefficient determination of quercetin was done by simple Shaking Flask method. 10 mg. of drug was dissolved in 10 ml. of phosphate buffer pH 7.2 system and 10 ml. of octanol in separating funnel. It was shaken well for 24 hours by orbital shaker then allowed to stand for complete phase separation. The concentration of drug was measured by UV spectrophotometric method. The remaining conc. of

sample in water phase was calculated by deduction from total amount of drug **(Lachman *et al* 1990).**

$\mathbf{P_{o/w}} = C_{oil}/C_{water}$

g) **Calibration of Quercetin**

Standard stock solution of quercetin was prepared by dissolving 100 mg drug in 100 ml methanol (i.e.1000µg/mL) and Methanol: PBS pH 7.4 (20: 80). Aliquot of desired concentration were prepared. The linearity was observed in the concentration range of 0.5-1.5 µg/mL for quercetin. The absorptivity coefficient of drug at desired wavelengths was determined.

i) Drug- Excipients Compatibility Studies

A small amount of drug substance with excipient that is, the physical mixture of the drug and excipient (in 1:1 ratio were prepared to have maximum likelihood interaction between them) was placed in a vial, and rubber stopper was placed on the vial and sealed properly. A storage period of 2 weeks at 60°C, and the same sample was retained for 2 months at 40°C. After storage the sample was observed physically for liquefaction, caking, odour or gas formation, discoloration (Saini and Gupta, 2009).

The drug–excipient interaction was also investigated using Silica gel–coated TLC (Thin Layer Chromatography) plates and a mixture of Chloroform: Methanol (9.5: 0.5). The TLC plates were prepared using slurry of Silica gel - G. The prepared plates were activated at 110° C for 15 min. On the activated plates, spots of each solution in methanol containing (a) Quercetin and (b) Quercetin containing a different experimental ratio of excipients, were applied. The R_f values were calculated from the chromatogram obtained and compared with the R_f values of quercetin alone (Arora and Mukherjee, 2002).

2.4 Development of Medicated Transdermal Patch

Transdermal patches were prepared by using solvent casting Technique. Matrix type transdermal patch consists of Polymer which was accurately weighed and stirred with suitable solvents Dichloromethane and methanol (1:1) by the solvent evaporation technique. Then Eugenol and Linseed oil was added as permeation enhancer, Polyethylene glycol used as plasticizer and Menthol as Counter irritant were added in the polymeric solution, mixed thoroughly by means of magnetic stirrer. To the above solution Quercetin, was added and poured into a petridish. It was covered with a funnel in inverted position. The solvent was allowed to evaporate at ambient conditions for 24 h. The patches were then covered with backing membrane cut into appropriate sizes, packed in aluminum foil and stored in desiccators. The so prepared films were sticked to the adhesive layer of bandage which was purchased from local market.

Table 1: Different Formulations of Transdermal Patch

Formulation	Q1	Q2	Q3	Q4	Q5	Q6
Quercetin (mg)	18	18	18	18	18	18
HPMC (mg)	25	50	75	100	125	150
Ethyl Cellulose(mg)	150	125	100	75	50	25
Eugenol (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Menthol (ml)	5%	5%	5%	5%	5%	5%
Linseed oil (ml)	3%	3%	3%	3%	3%	3%
Poly Ethylene Glycol	3 %	3 %	3 %	3 %	3 %	3 %
Solvent	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

2.5 Evaluation of Transdermal Patch

The physical parameters such as thickness, weight variation, folding endurance of various films were determined.

2.5.1 Physical appearance

All the prepared patches were visually inspected for color, clarity, flexibility, and smoothness.

2.5.2 Weight Variation: Uniformity of weight was determined by weighing five matrices of each formulation. Each film unit was weighed individually on a digital balance, the average weight of film was taken as the weight of the film **(Hull M S *et al* 2002).**

2.5.3 Thickness uniformity*:* The thickness of the films was determined by measuring the thickness at five sites on three films of each formulation using digital Vernier calipers and the average was calculated **(Hull M S, 2002 and Krishnaiah Y. S, 2004)**

2.5.4 Folding Endurance: The folding endurance is expressed as the number of film folded at the same place to break the specimen or to develop visible cracks. Three films of each formulation of size were cut by using sharp blade. The mean value of triplicate and standard deviation were calculated **(Krishnaiah Y. S *et al* 2004).**

2.5.5 Flatness: A transdermal patch should possess a smooth surface and should not constrict with time. This can be demonstrated with flatness study. For flatness determination, one strip is cut from the center and two from each side of patches. Zero percent constriction is equivalent to 100 percent flatness.

$$\% \text{ constriction} = \frac{L1-L2 \times 100.}{L1}$$

L2 = Final length of each strip

L1 = Initial length of each strip

2.5.6 Surface pH determination: For the determination of surface pH of the patch a small area of the film was cut and was allowed to swell by keeping it in distilled water for 1 h in glass tubes. The surface pH was then noted by bringing a combined glass electrode near the surface of the film and allowing it to equilibrate for 1 min.

2.5.7 Water vapor absorption: The percent moisture absorption test was carried out to check the physical stability and integrity of the films in high humid conditions. The prepared films (3.14 cm^2) were individually weighed accurately and exposed to 85 ± 5% relative humidity in a desiccator containing 100 ml of saturated solution of potassium chloride at room temperature. During this period, the films were weighed at regular time intervals of 24, 48, and 72 h. The percent moisture absorption was determined from the following formula:

% moisture uptake =(Final weight – Initial weight) × 100/Initial weight

2.5.8 *In-vitro* Permeation Studies: An *in vitro* permeation study was carried out by using Franz diffusion cell. Full thickness abdominal skin of male Wistar rat weighing 200 to 250 g was used. Hair from the abdominal region was removed carefully by using an electric clipper; the dermal side of the skin was thoroughly cleaned with distilled water to remove any adhering tissues or blood vessels, equilibrate for an hour in phosphate buffer pH 7.4

before starting the experiment, and was placed on a magnetic stirrer with a small magnetic needle for uniform distribution of the diffusants. The temperature of the cell was maintained at 32 ± 0.5◦C using a thermostatically controlled heater. The isolated rat skin piece was mounted between the compartments of the diffusion cell, with the epidermis facing upward into the donor compartment. Sample volume of 5 mL was removed from the receptor compartment at regular intervals, and an equal volume of fresh medium was replaced. Samples were filtered through whatmann filter and were analyzed using Shimadzu UV 1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan). Flux was determined directly as the slope of the curve between the steady-state values of the amount of drug permeated ($mg*cm^2$) versus time in hours and permeability coefficient was deduced by dividing the flux by the initial drug load ($mg*cm^2$).

2.6 Preliminary Pharmacological Screening

The Pharmacology Screening was performed in Modern Institute of Pharmaceutical Sciences approved by CPCSEA (Approval No.: **1509/PO/RE/S/11CPCSEA**).

2.6.1 Acute Dermal Toxicity Study

Healthy young albino rats, were used as the experimental animals were acclimatized to the laboratory conditions for at least 5 days prior to the test, according to Acute dermal toxicity, Section No. 402. Before the test, animals were randomized and assigned to the treatment groups. Approximately 24 hours before the test, fur were removed from the dorsal area of the trunk of the test animals by clipping or shaving. Care was taken to avoid abrading the skin. Different formulations of transdermal patches, as the test substance, were applied to an area of skin. The patch was loosely held in contact with the skin for 4 hours and were then removed. Observations were recorded an hour after the removal of the patch. No clinical signs of dermal toxicity were observed in any of the animals treated with the test substance upon repeated application of the transdermal patch for up to 28 days **(Acute dermal toxicity, 402)**

2.6.2 **Anti-inflammatory activity**

Anti-inflammatory activity was assessed by carrageenan induced rat paw edema method as per the procedure described elsewhere by Winter *et al.,* 1962. Albino rats of either sex weighing 200 –250 gm were divided in 8 groups (n=6). Group-I received 0.5% CMC suspension (control), Group- II, III and IV, V, VI, VII applied transdermal patch of different formulation Q1, Q2, Q3, Q4, Q5 and Q6 respectively at abdominal region after depilating the abdominal region. Group- VIII standard group NU Patch 200mg (**Nilufer Ercan, *et al* 2013)**. Animals were treated with transdermal patch and subsequently 1 h after treatment. 0.1ml of 1% suspension of carrageenan in normal saline was injected into the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 0, 1, 2, 3 and 4hr after carrageenan injection using digital paw edema meter.

The inhibition of inflammation was calculated using the formula,

% inhibition = 100 (1-Vt/Vc),

Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

2.6.3 Anti-inflammatory activity by Xylene induced mouse ear edema model

The effect of different transdermal patch on acute edema was assessed by using xylene induced ear edema in mice. Male Swiss albino mice weighing 18-27 g were divided in 8 groups (n=6). Group-I received 0.5% CMC suspension (control), Group- II, III and IV, V, VI, VII applied transdermal patch of different formulation Q1, Q2, Q3, Q4, Q5 and Q6. Group-VIII standard group were applied the patch of NU Patch 200mg (**Nilufer Ercan, *et al* 2013)**. One hour after application of transdermal patch and, 50ml of Xylene was applied to the anterior and posterior surfaces of the right ear under light ether anesthesia. The left ear was considered as control. Four-hour later xylene application mice were sacrificed by cervical dislocation and both ears were removed. Earlobes were punched out in circular disc using metal punch (6 mm diameter) and weighed. The difference in the weight of discs from right treated and left untreated ear was calculated and was used as measure of edema.

The difference in the weight of the discs from the right treated and left untreated ears was calculated and used as a measure of edema (Tubaro et al., 1985; Atta and Alkohafi, 1998). The level of inhibition (%) of edema was calculated using the relation:

Inhibition (%) = 100[1-(Et/Ec)] where

Et= Average edema of the treated group; Ec= Average edema of the control group

3.0 RESULTS AND DISCUSSIONS

3.1 Preformulation Studies

The preformulation study was performed to assure the authenticity of sample drug and determination parameters for development of Transdermal patch.

3.1.1 Identification of Drug

a) Organoleptic properties

Organoleptic characteristics of the drug were found within standard limits as shown in Table. 3.1

Table 2: Physical Properties of The Drug

S. No.	Properties	Inference
1	Color	Yellowish
2	Odor	Odorless
3	Taste	Tasteless
4	State	Crystalline Powder

b) Melting Point

The melting point of drug sample (Quercetin) was found to be 316.76 which compared with reported value (310^0C - 320^0C) indicated that the drug sample was pure.

c) UV absorption spectra of Quercetin

The maximum absorbance of drug in methanol was found to be at λ_{max} 256 nm which is matched with reference indicated that the drug sample was pure.

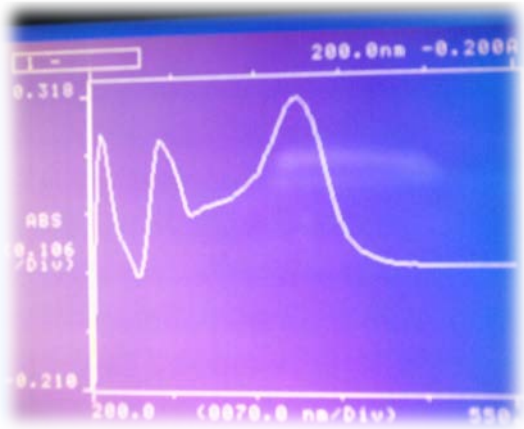

Figure 1: Ultraviolet Absorption Maxima of Quercetin

d) Fourier Transform Infra-Red analysis

The FTIR analysis of the sample was carried out for compound identification. The powdered drug was placed carefully over sample holder ensuring no air entrapment, thereafter the sample was scanned. The FTIR spectrum for pure quercetin is shown in Figure 6.2 where its characteristic bands were detected. OH groups stretching were detectable at 3406 and 3283 cm−1, whereas OH bending of the phenol function was detectable at 1379 cm−1. The C=O aryl kenotic stretch absorption was evident at 1666 cm−1. C=C aromatic ring stretch bands were detectable at 1610, 1560, and 1510 cm−1. The in-plane bending band of C–H in aromatic hydrocarbon was detectable at 1317 cm−1, and out-of-plane bending bands were evident at 933, 820, 679, and 600 cm−1. Bands at 1263, 1200, and 1165 cm−1 were attributable to the C–O stretching in the aryl ether ring, the C–O stretching in phenol, and the C–CO–C stretch and bending in ketone, respectively

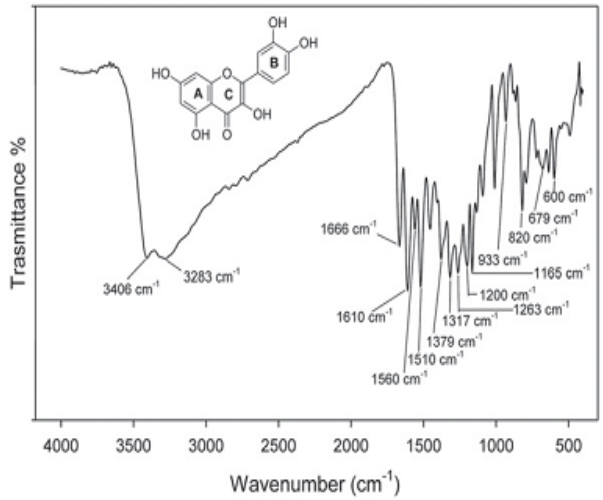

Figure 2: Infrared Spectrum of drug

e) Determination of Solubility

The solubility study revealed that the drug sample was freely soluble in methanol and Methanol : PBS pH 7.4 (10 : 90), sparingly soluble in Ethanol , PBS (pH 7.2) and Methanol : PBS pH 7.4 (05 : 95), slightly soluble in Water.

Table 3: Solubility of quercetin

S. No.	Solvent	Quercetin
1	Ethanol	+++
2	Methanol	+++++
3	Water	++
4	PBS (pH 7.2)	+++
5	Methanol : PBS pH 7.4 (05 : 95)	+++
6	Methanol : PBS pH 7.4 (10 : 90)	++++
7	Methanol : PBS pH 7.4 (20 : 80)	+++++

++++++ = Very soluble <1 part; +++++ = Freely soluble 1-10 part; ++++ = Soluble 10-30 parts; +++ = Sparingly soluble 30-100 parts; ++ = Slightly soluble 100-1000 parts;+ = Very slightly soluble 1000-10000 parts; – = Practically insoluble >10000 parts

f) Determination of Partition Coefficient

Partition coefficient was determined in Octanol /phosphate buffer pH 7.2 system and was found to be 0.98. This study revealed the hydrophobic nature of quercetin and further indicated that it is a suitable candidate for transdermal drug delivery system.

g) Preparation of Calibration Curve of Quercetin

Standard stock solution of quercetin was prepared by dissolving 100 mg drug in 100 ml methanol (i.e.1000µg/mL) and Methanol: PBS pH 7.4 (20:80). Aliquot of desired concentration were prepared. The absorptivity coefficient of drug at desired wavelengths was determined.

Table 4: Calibration of quercetin at 256 Nm Λmax in methanol

S.No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	2	0.218
3.	4	0.445
4.	6	0.614
5.	8	0.802
6.	10	0.912

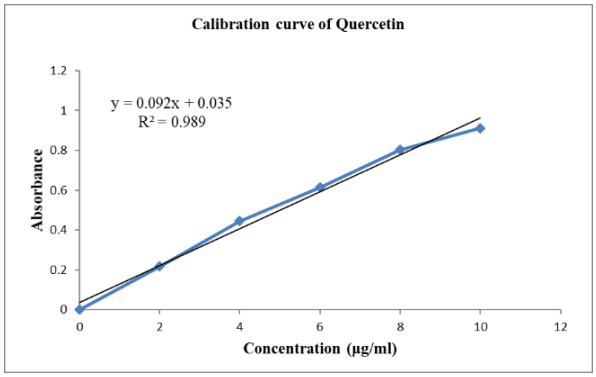

Figure 3: Calibration curve of quercetin in methanol

h) Drug- Excipients Compatibility Studies

Quercetin containing different experimental ratio of excipients, were applied. The R_f values were calculated from the chromatogram obtained and compared with the R_f values of quercetin alone (Arora and Mukherjee, 2002)

3.2 Evaluation f Transdermal Patch

3.2.1 Physical Parameters

The physical parameters such as thickness, weight variation, folding endurance of various films were determined. All the films were evaluated for their physical parameters (weight, thickness, folding endurance, flatness, and surface pH), and they were found to be flexible, uniform, smooth, and transparent (Table 6). All the formulations were uniform in their weight, thickness, folding endurance, and diameter, with low SD values. The weight of the prepared transdermal patches for different type of formulations ranged between 210.70 ± 4.01mg and 218.90 ± 2.45 mg, but within a formulation, all the patches showed low standard deviation values. The thickness of the patches varied from 0.049 ± 0.001mm to 0.054 ± 0.001 mm. Low standard deviation values in the film thickness measurements ensured uniformity of the patches which further indicated the reproducibility of the procedure followed for the preparation of the patches. Folding endurance values varied between $298.3 \pm 2.08$and 378.1 ± 2.31. The flatness study showed that all the formulations had the same strip length before and after their cuts, indicating 100% flatness. Thus, no amount of constriction was observed which indicated that all patches had smooth flat surface which would be maintained when the patches are applied to the skin. The surface pH of the prepared transdermal patches for different type of formulations was found to be in the range of 5.43 and 5.71. The surface pH of which indicated the absence of skin irritancy. No significant changes in pH value were observed during the study.

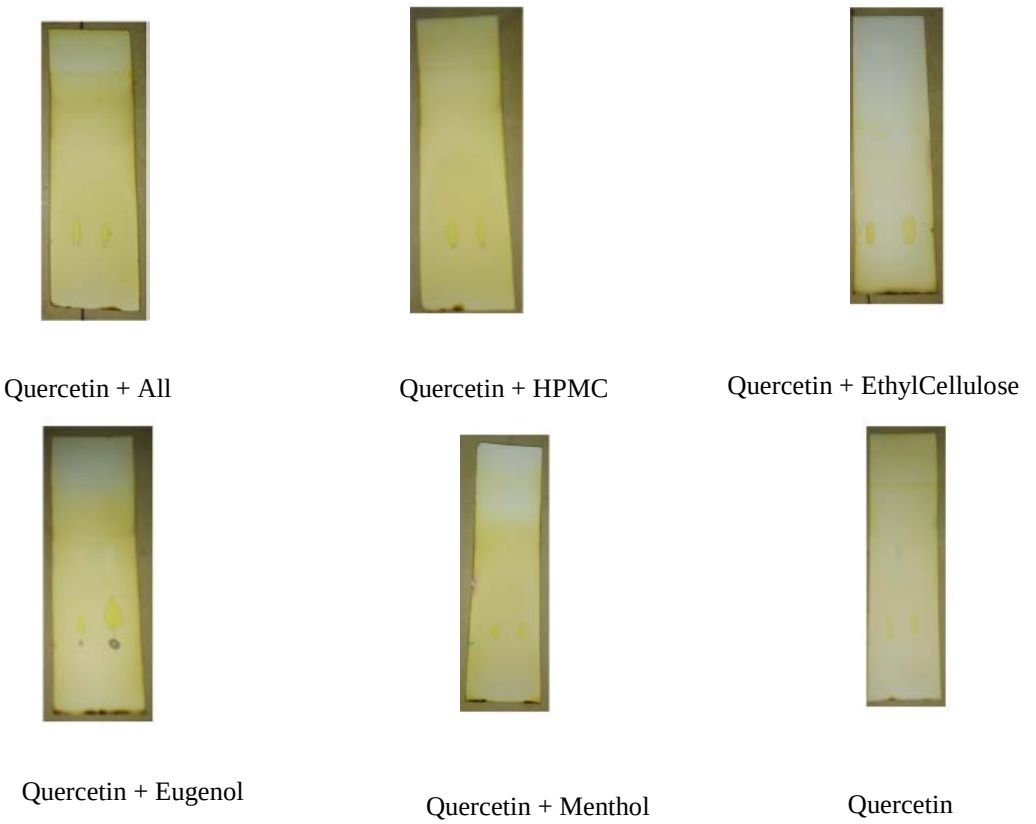

Figure 4: Drug Interaction Studies

Table 5: Interaction Study

S. No.	Parameter	$R_{f\ (Initial)}$	$R_{f\ (After\ 4\ week)}$	Observation
1	Quercetin	0.438	0.437	As no changes in R_f value was observed hence it shows no interaction after 4 weeks
2	Quercetin + HPMC	0.511	0.514	
3	Quercetin + Ethyl Cellulose	0.524	0.526	
4	Quercetin + Eugenol	0.522	0.524	
5	Quercetin + Menthol	0.523	0.526	

Table 6: Physical parameters of transdermal patch

Code	Weight(mg) ± SD	Thickness (mm) ± SD	Folding endurance ± SD	Flatness (%)	Surface pH
Q1	215.30 ± 2.68	0.053 ± 0.001	306.3 ± 2.52	100	5.43
Q2	212.10 ± 2.48	0.048 ± 0.001	304.6 ± 2.51	100	5.66
Q3	210.70 ± 4.01	0.049 ± 0.001	378.1 ± 2.31	100	5.47
Q4	218.90 ± 2.45	0.053 ± 0.002	302.6 ± 2.08	100	5.67
Q5	216..10 ± 2.77	0.050 ± 0.001	298.3 ± 2.08	100	5.52
Q6	215.80 ± 2.74	0.054 ± 0.001	308.6 ± 2.08	100	5.71

Values are expressed as mean □SD, n = 3

Table 7: Water vapor absorption studies of transdermal patch

Code	Average initial weight of Patch (mg)	Weight of Patch			Total Moisture Gain	% Moisture Absorption	WVA rate = *WL/S*
		Day 1	Day 2	Day 3			
Q1	218.43±2.10	218.67	218.89	219.17	0.74 ± 0.006	0.3516 ±0.003	0.003161
Q2	213.33±1.72	2131.65	213.91	214.23	0.9 ± 0.007	0.4537 ±0.003	0.003217
Q3	210..26±2.01	210.31	210.56	210.64	0.38 ± 0.003	0.1798 ±0.001	0.001386
Q4	218.19±2.13	218.57	218.91	219.24	1.05 ± 0.01	0.4282 ±0.004	0.004349
Q5	214.31±2.10	214.76	214.87	215.09	0.78 ± 0.006	0.3762 ±0.003	0.002955
Q6	219.11±2.17	219.67	219.81	220.1	0.99 ± 0.008	0.4072 ±0.003	0.004051

Values are expressed as mean □SD, n = 3

Table 8: Cumulative percentage release of quercetin

Code	**Cumulative % release of drug**										
	1 h	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**	**7 h**	**8 h**	**9 h**	**10 h**	**24 h**
Q1	0.95	1.81	2.26	5.58	7.24	8.97	9.80	13.50	14.03	15.46	57.02
Q2	0.29	0.44	3.46	5.01	6.04	7.67	8.92	10.40	11.87	13.79	52.66
Q3	4.65	6.20	10.29	12.17	14.45	16.01	18.29	24.50	28.26	30.87	85.77
Q4	2.69	6.01	8.07	9.96	11.66	12.92	14.54	16.60	19.56	21.62	74.78
Q5	1.66	2.57	5.56	7.30	8.88	11.64	12.87	13.86	16.85	17.19	64.27
Q6	2.78	4.21	4.80	6.57	9.2	11.21	12.99	13.92	15.77	17.37	48.08

3.2.2 Water vapor absorption studies

The prepared patches showed minimal moisture absorption rates ranging from 0.001386 to 0.004051% thus ensuring general stability and protection from microbial contamination. and increase in the HPMC concentration increased the moisture absorption capacity. Therefore, formulation Q3 which is having HPMC (75 mg) and ethyl cellouse (100 mg) showed significantly less water absorption.

3.2.3 *In vitro* drug release studies

The drug release characteristics of the formulation were studied *in vitro* conditions by using rat skin membrane. The formulation Q1, Q2, Q3, Q4, Q5 & Q6 has shown release of about 57.02%, 52.66%, 85.77%, 74.78%, 64.27%, and 48.08% at 24 h and respectively. The order of drug release was found to be Q3>Q4>Q5>Q1>Q2>Q6.

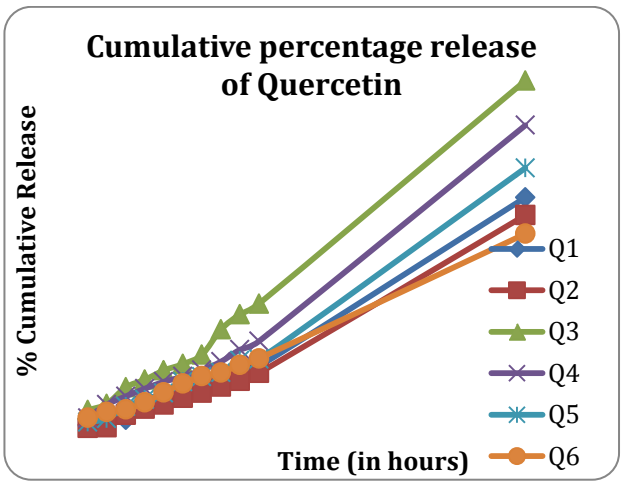

Figure 5: Cumulative percentage release of quercetin

3.2.4 Pharmacological Screening

a) Acute Dermal Toxicity Study

Wistar albino rats were divided into two groups (control and transdermal patch-treated group). Total of six rats/sex/group were used

Clinical observation and mortality. None of the animals showed any clinical signs, and none showed any overt signs of toxicity from the first day until the end of the experiment. The skin of the animals appeared normal, and no erythema or edema was noted. The locomotor behavior was also normal, and there were no signs of toxicity detected in the treated rats.

Terminal body weight trends. In this study, no treatment related changes were noted in the terminal body weights of rats when compared to their negative control counterparts. There were no statistically significant mean weight differences in body weights between the control and the treated groups from the first day of patch application through the end of the experiment.

Necropsy and organ weight. Following necropsy, no macroscopic changes were observed in the internal organs of all treated animals. The absolute and relative organ weights of rats showed no statistically significant difference between test and control groups.

Histopathology. Analysis of the toxic potential of a chemical agent on target organs is incomplete without gross and histopathological evaluation. Histopathological examination of selected organs of both treated and control animals showed normal architecture, suggesting no abnormal findings in the histological evaluation.

Skin irritation studies

The skin irritation study reveals that the drug loaded and unloaded patches didn't cause any noticeable signs of irritation or oedema on albino rat's skin, indicating the skin compatibility of drug as well as polymer matrix.

b) Anti-inflammatory activity of transdermal patch

Results of the carrageenan induced rat paw edema method is presented in Table 10. It demonstrates that Quercetin transdermal patch exhibited significant anti-inflammatory activity in the later phase of the carrageenan-induced paw edema test. The paw volume in the control group prominently increased after intraplantar injection of carrageenan. Various formulation of transdermal patch i.e Q1, Q2, Q3, Q4, Q5, Q6 showed significant decrease in paw edema volume. Out of six formulation showed Q3 reduced the paw edema in 4th hour to 0.24±0.020** which was found to be highly significant when compared to control 0.69±0.069 and standard Nu Patch 200 mg i.e 0.20±0.024** In our experiment, Q3 caused a potent inhibition of the inflammation at the fourth hour. Therefore, it may inhibit the synthesis of prostaglandins in the late phase of inflammation. PGs are hormone-like endogenous mediators of inflammation and formed from arachidonic acid by COX-1 and the inducible form COX-2.

Anti-inflammatory activity of Transdermal Patch by Xylene induced mouse ear edema model

Xylene-induced ear edema test to evaluate the topical anti-inflammatory effect. As shown in Table 11, Various formulation of transdermal patch i.e Q1, Q2, Q3, Q4, Q5, Q6 showed significant decrease the ear edema rate by 31.16% and the edema rate was smaller than that of Nu Patch 200 mg (30.26 %). Both formulation Q3 and Nu Patch 200 mg inhibited markedly the ear edema compared to the control.

Table 9: Estimation of body weight

Group	Sex	Terminal Body Weight (g) Mean ±SD		
		0 day	7 days	14 days
Control	Male	222.87 ± 9.53	231.37 ±8.32	245.75 ± 9.88
	Female	210.49 ± 7.30	211.15 ± 5.48	212.32 ± 6.37
Treated	Male	219.06 ± 7.87	227.44 ± 9.31	248.68 ± 10.22
	Female	216.98 ± 6.34	204.56 ± 7.35	216.72 ± 6.21

Table 10: Anti-Inflammatory activity of transdermal patch by carrageenan induced rat paw edema method

Group	No. of animals	Treated	Dose mg/kg	Paw edema volume (ml)			
				1 hr	2 hr	3 hr	4 hr
Group I	6	Control	0.5% CMC	0.70±0.074	0.70±0.074	0.70±0.071	0.69±0.069
Group II	6	Q1	TDP	0.70±0.036*	0.68±0.026*	0.64±0.017*	0.59±0.017*
Group III	6	Q2	TDP	0.69±0.036**	0.66±0.027**	0.52±0.016**	0.50±0.014*
Group IV	6	Q3	TDP	0.70±0.020*	0.64±0.028*	0.45±0.028*	0.24±0.020**
Group V	6	Q4	TDP	0.70±0.041*	0.66±0.039*	0.59±0.036*	0.57±0.042*
Group VI	6	Q5	TDP	0.70±0.041*	0.67±0.039*	0.61±0.036*	0.56±0.042*
Group VII	6	Q6	TDP	0.70±0.041*	0.64±0.039*	0.60±0.036*	0.55±0.042*
Group VIII	6	Nu Patch 200 mg	200 mg	0.70±0.017**	0.60±0.017**	0.40±0.015**	0.20±0.024**

Values are expressed as mean □SEM, n = 6 rats in one group. ns = not significant * $p < 0.05$, ** $p < 0.01$, & *** $p < 0.001$, One-way ANOVA followed by Dunnet's Test.

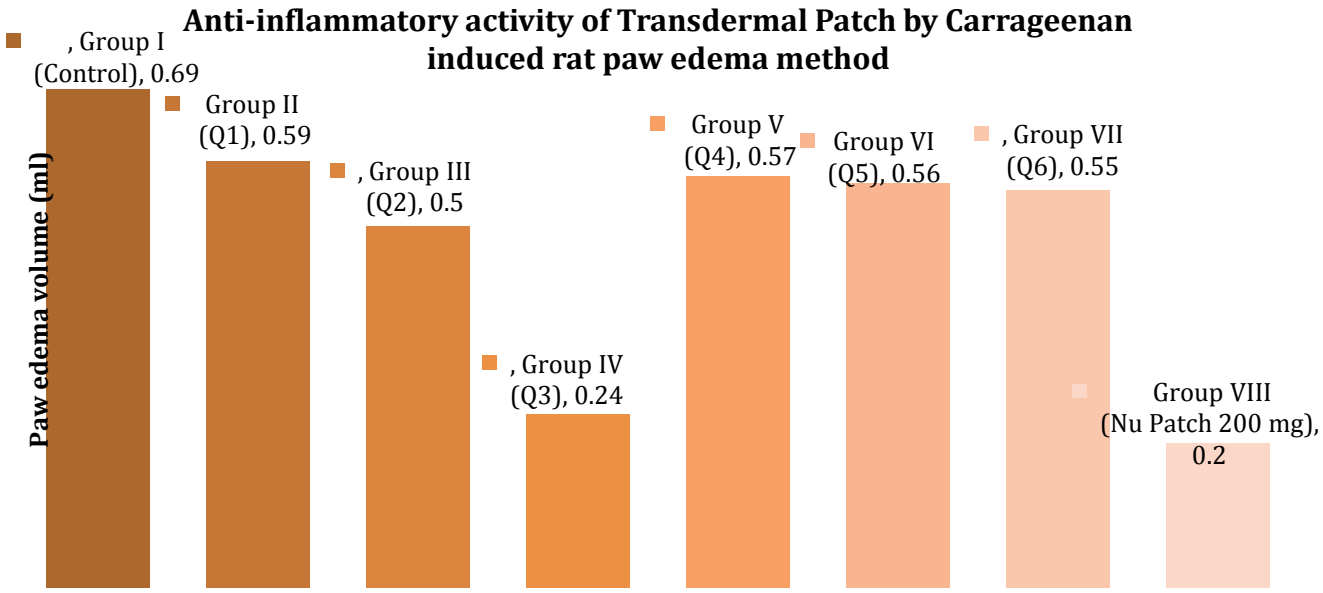

Figure 6: Anti-Inflammatory activity of transdermal patch by carrageenan induced rat paw edema method

Table 11: Anti-Inflammatory activity of transdermal patch by xylene induced mouse ear edema model

Group	No. of animals	Treated	Dose mg/kg	Weight of Ear		Edema Rate (%)
				Weight of Left Ear (mg)	Weight of right Ear (mg)	
Group I	6	Control	0.5% CMC	0.76±0.05	1.43±0.21	88.15
Group II	6	Q1	TDP	0.76±0.08	1.33±0.17*	75.15*
Group III	6	Q2	TDP	0.78±0.08	1.23±0.15*	57.69*
Group IV	6	Q3	TDP	0.77±0.08	1.01±0.20***	31.16***
Group V	6	Q4	TDP	0.76±0.08	1.27±0.22*	67.10*
Group VI	6	Q5	TDP	0.78±0.08	1.30±0.14*	66.66*
Group VII	6	Q6	TDP	0.77±0.08	1.38±0.16	79.22
Group VIII	6	Nu Patch 200 mg	200 mg	0.76±0.09	0.99±0.14***	30.26**

Values are expressed as mean ☐SEM, n = 6 rats in one group. ns = not significant * $p < 0.05$, ** $p < 0.01$, & *** $p < 0.001$, One-way ANOVA followed by Dunnet's Test.

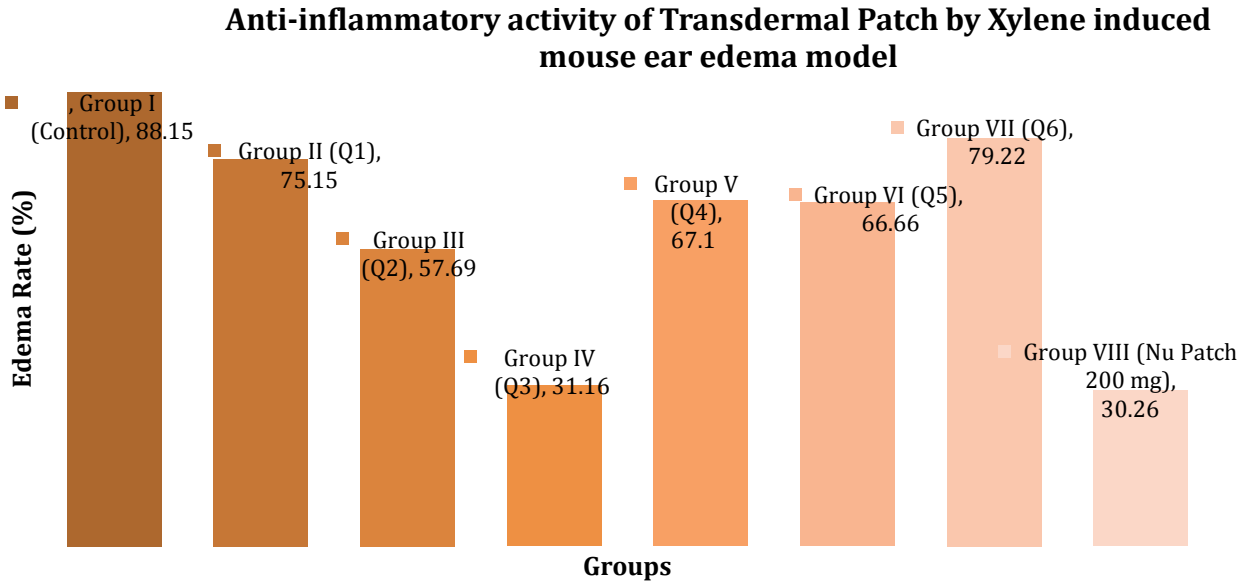

Figure 7: Anti-inflammatory activity of transdermal patch by xylene induced mouse ear edema model

4.0 CONCULSION

The transdermal patch of Quercetin was prepared successfully by solvent casting method. In conclusion, the present data confirm the feasibility of developing Quercetin transdermal patches on an industrial scale.

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