IJP (2015), Vol. 2, Issue 8 **(Research Article)**

Received on 17 June 2015; received in revised form, 15 July 2015; accepted, 14 August 2015; published 31 August 2015

IN-VITRO, EX-VIVO SKIN PERMEATION AND BIOLOGICAL EVALUATION OF 18-β-GLYCYRRHITIC ACID TRANSDERMAL PATCHES

Prachi M. Patel [*1] and Vh Bhaskar [2]

Department of Pharmacognosy [1], PHC, Surendranagar, Ahmedabad - 380006, Gujarat, India.
Department of Medicinal Chemistry [2], Gahlot Institute of Pharmacy, Navi Mumbai - 400709, Maharashtra, India.

Keywords:

Reservoir-type Transdermal patch, GA, Piperine, Carbopol 934, Skin irritation test, Inhibition of edema

Correspondence to Author:
Prachi M. Patel

M. Pharm,
Department of Pharmacognosy,
PHC, Surendranagar, Ahmedabad - 380006, Gujarat, India.

E-mail: prach_patel@yahoo.com

ABSTRACT: Objective: a Transdermal patch is a promising approach that allows continuous input of drugs with short biological half-lives. 18 β-Glycyrrhizic acid (GA) is chemically pentacyclic triterpenoid which possesses powerful anti-inflammatory action. The present study was designed to formulate GA reservoir-type transdermal patches using piperine as bioenhancer. **Method:** Transdermal Patch of GA was prepared using heat seal technique using 2^3 factorial design allowing for independent variables like penetration enhancers, formulation matrix and rate controlling membranes. The GA patches were evaluated for *in-vitro* and *ex-vivo* drug permeation through cellophane membrane and rat skin respectively. The biological evaluation regarding skin irritation study and the anti-inflammatory effect of GA patches on rat paw edema were tested and compared with standard Aceclofenac patch. **Result:** The reservoir-type patch of F4 formulation, containing 5% menthol as a permeation enhancer, 42% ethanol, 2% carpool 934 gel bases (50 g) with 0.5% piperine provided an improved sustained release of phytopharmaceuticals through transdermal administration. The anti-inflammatory action F4 formulation showed no skin irritation with significant inhibition of rat paw edema compared with standard patch. **Conclusion:** The GA patch was demonstrated the feasibility for future biopharmaceutical study in clinical trials.

INTRODUCTION: GA is a pentacyclic triterpenoid derivative of the beta-amyrin type obtained from the hydrolysis of glycyrrhizic acid, which was obtained from the herb liquorice. The structure of GA is similar to that of cortisone. Both molecules are flat and identical to position 3 and 11.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.2(8).400-08

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(8).400-08

This might be the basis for licorice's anti-inflammatory action. It is solid off-white powder, molecular formula C30H46O4, melting point 292 ºc, molar mass 470.68 g mol^{-1}, soluble in ethanol and chloroform 1, 2. 100 µM GA suppresses LPS-induced TNF-α production and NF-κβ activation in mouse macrophages. The results showed that treatment with 20–75 µM GA inhibit the production of LPS-induced nitric oxide (NO), prostaglandin E_2 (PGE_2) and intracellular reactive oxygen species (ROS) [3]. The liposomal gel with GA 0.9% showed stronger anti-inflammatory activity than triamcinolone acetonide and econazole nitrate cream [4].

The oral LD_{50} in rats was reported to be 610 mg/kg. Higher LD_{50} values were generally reported for salts [5]. Piperine is a pungent alkaloid present in *Piper nigrum* Linn, was reported to have antidiarrhoeal, anti-inflammatory, immune-enhancing, anticonvulsant and antioxidant activity. Piperine may work as a drug bioavailability enhancer, which is evident by the increase in the C_{max} and AUC of phenytoin, propranolol, and theophylline by co-administration of piperine (20 mg/kg) [6]. Transcutaneous permeation of Repaglinide (antidiabetic drug) in rats was enhanced by 8-fold from transdermal formulations containing piperine (0.008% w/v) [7]. Piperine enhances bioavailability by inhibiting various metabolizing enzymes as well as transdermal permeation of aceclofenac via partial extraction of stratum corneum (SC) lipid and interaction with SC keratin. This provides a scientific basis for the use of Piperine to enhance the therapeutic efficacy of the concurrently administered drugs [8, 9].

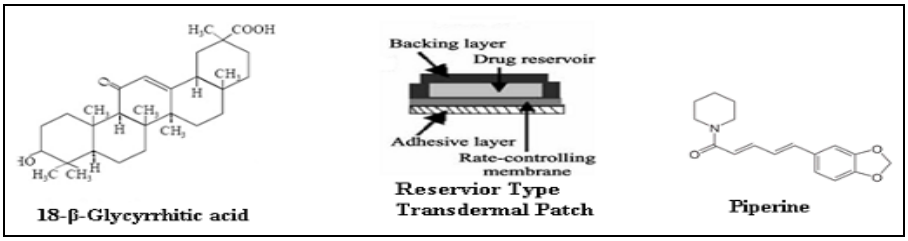

FIG. 1: RESERVOIR TYPE TRANSDERMAL PATCH

The objective of the present study was to design and evaluate reservoir type transdermal patch of GA with Piperine as bioenhancer to avoid the hepatic first-pass metabolism and improve the therapeutic efficacy of the drug.

MATERIAL AND METHODS:

Materials: GA and Piperine were purchased from Yucca Enterprises, Mumbai-37. The Carbopol 934, Benzyl alcohol, Menthol, Alcohol, Triethanolamine and polyisobutylene were procured from Chem dyes corporation, Ahmedabad. Ethylene vinyl acetate (EVA) membranes with 9% VA content (EVA9%; 3M CoTran 9702) and 19% VA content (EVA19%; 3M CoTran 9715) were gift samples from 3M Pharmaceuticals, USA. The backing layer (perlite-polyester film) and the release liner (pedlite-polyester film). All other chemicals and solvents used were of analytical grade.

Methods: 4 g of Carbopol 934 was dispersed in100 ml of distilled water and kept overnight to get a smooth gel. Benzyl alcohol as a preservative was added into gel base. Menthol, 18-β-glycyrrhetic acid and Piperine were dissolved in ethanol and poured into the Carbopol 934 gel base with continuous stirring. Triethanolamine was added dropwise to the formulation for adjustment of required skin pH (6.8-7) mentioned in **Table 1, 2**.

TABLE 1: FORMULATION OF TRANSDERMAL PATCHES OF GA (2^3 FACTORIAL DESIGN)

S. no.	Ingredients	Formulation of gel base / Formulations							
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
1	Carbopol 934	4%	4%	4%	4%	4%	4%	4%	4%
2	Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
	Formulation of medicated gel								
1	Gel base	50%	50%	50%	50%	60%	60%	60%	60%
2	Benzyl alcohol	1%	1%	1%	1%	1%	1%	1%	1%
3	18 ß-glycyrrhetinic acid	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
4	Piperine	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
5	Menthol	2%	2%	5%	5%	2%	2%	5%	5%
6	Alcohol	45%	45%	42%	42%	35%	35%	32%	32%
7	Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
	Formulation of Reservoir TDDS								
1	EVA with % VA	9%	19%	9%	19%	9%	19%	9%	19%

TABLE 2: FORMULATION OF TRANSDERMAL PATCHES OF GA SHOW BIOENHANCER PROPERTY OF PIPERINE

S. no.	Ingredients	Formulations			
		F9	F10	F11	F12
Formulation of gel base					
1	Carbopol 934	4%	4%	4%	4%
2	Distilled water	100 ml	100 ml	100 ml	100 ml
Formulation of medicated gel					
1	Gel base	50%	50%	50%	50%
2	Benzyl alcohol	1%	1%	1%	1%
3	18 ß-glycyrrhetinic acid	0.9%	0.9%	0.9%	0.9%
4	Piperine	-------	0.25%	1%	0.5 %
5	Menthol	5%	5%	5%	--------
6	Alcohol	42.5%	42.25%	41.5%	47%
7	Triethanolamine	q.s	q.s	q.s	q.s
Formulation of Reservoir TDDS					
1	EVA with % VA	19%	19%	19%	19%

Accurately weighed the quantity of the 1g gel (9 mg GA) was placed on a sheet of the backing layer (pedlite-polyester film) covering 2 cm × 2 cm areas. A rate-controlling membrane (EVA with 9% or 19% vinyl acetate) was placed over the gel, and the edges of 2 cm × 2 cm area was heat-sealed to obtain a leak-proof device. To ensure intimate contact of the patch to the skin, a pressure sensitive adhesive, polyisobutylene (PIB), was applied onto rate controlling membrane (3 ml; 10% w/v in petroleum ether). A release liner was placed over the adhesively coated rate controlling membrane [10].

Preformulation Studies:

Description: 18-β-glycyrrhetic acid was physically examined for color and odor.

Solubility: The solubility of 18-β-glycyrrhetic acid was determined in water, ethanol, chloroform, phosphate buffer 7.4, DMSO.

Drug-Polymer Interaction Study: The FTIR spectra (Spectrometer Model 2500) was taken and analyzed for any interaction between the drug and the polymers.

Evaluation of Transdermal Patches:

Thickness: Three patches from each batch were taken, and the weight of each patch was found by using electronic balance. The the average weight of a single patch was determined [11].

Weight Variation: The thickness of the patch was assessed by using screw gauge at different points of the patch. From each formulation three, randomly selected patches were used. The average value for the thickness of a single patch was determined [11].

Drug Content Uniformity: The patch (4 cm^2) was added to a beaker containing 100 ml of phosphate buffered saline pH 7.4 (PBS). The medium was stirred (50 rpm) with a magnetic bead for 24 h. The contents were filtered using what man filter paper and 0.5 ml filtrate were extracted with 5 ml chloroform. Chloroform layer evaporated on a water bath. Then reconstituted in 5 ml methanol and analyzed for GA and Piperine with maximum absorption at 250 nm and 342.5 nm using simultaneous UV determination against the reference solution consisting of placebo patch [11].

***In-vitro* Drug Release:** The fabricated patch was placed on cellophane membrane (cellulose acetate membrane) and attached to the Franz diffusion cell such that the cell's drug releasing surface towards the receptor compartment which was filled with 50 ml of Phosphate buffer pH 7.4 at 37 ± .5°. The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was constantly stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.5 °C. The aliquots (5 ml) were withdrawn at predetermined time intervals and replaced with the same volume of Phosphate buffer pH 7.4. The aliquot (5ml) was extracted with 5 ml chloroform. Chloroform layer evaporated on the water bath.

Then reconstituted in 5 ml methanol. The samples were analyzed for GA and Piperine with maximum absorption at 250 nm, and 343.17 nm using simultaneous UV determination against the reference solution consisting of a placebo patch showed in **Fig. 2** and **Table 3** [11].

***Ex-vivo* Permeation Studies Using Rat Skin:** Albino rats of Wistar strain of either sex between 200-210 g breed were selected for the studies. The animals were fastening overnight but allowed water *ad libitum*. The permission to carry out animal studies was obtained from CPCSEA [Reg. no/date: No. 1338/C/CPCSEA, 07/04/2010]. Hairs from the abdominal side of albino rats were removed by shaving. The animals were sacrificed. The full thickness skin of the abdomen was surgically removed, and the adhering subcutaneous fat was carefully cleaned. Any trace of fat adhering was finally removed by wiping with cotton soaked in isopropyl alcohol. Finally, skin rinsed with distilled water then with saline [12].

The transdermal permeation was performed in Franz Diffusion cell. The cells were filled with freshly prepared phosphate buffer pH 7.4. While placing the patch, the donor compartment contains patch on stratum corneum side of skin and dermis side was facing receptor compartment. Receptor compartment contains phosphate buffer pH 7.4 and samples were withdrawn at regular time intervals and replaced the same with receptor fluid. The samples were analyzed for GA and Piperine with maximum absorption at 250nm and 343.17 nm using simultaneous UV determination against the reference solution consisting of placebo patch [13].

Biological Evaluation of GA Transdermal Patch:

Skin Irritation Test: The irritancy of the selected patches was evaluated in terms of biological investigation on male albino rats (200 -210 g) based upon the method described by Draize et al. (1944) [14]. The rats have anesthetized with thiopental (60mg/kg) injection (i.p) then the dorsal side of the rat will be shaved with clippers 24 h before the beginning of the experiment. The animals were divided into 3 groups each group consists of 6 rats: Group A was served as control. Group B was received 0.5ml of a 0.8% v/v aqueous formalin solution as a standard irritant [15]. Group D (GA with Piperine) was received medicated patches for 3 days (A new patch will be applied daily). The application sites were examined for edema and erythema after 1, 12, 24 and 72 h., then graded (0-4) according to a visual scoring scale always by the same investigator; the final score will represent the average of the 24 and 72 h readings. The erythema scale is as [14]: 0, none; 1, slight; 2, well defined; 3, moderate; and 4, scar formation. The edema scale is: 0, none; 1, slight; 2, well defined; 3 moderate; and 4, severe.

The primary irritancy index (PII) will be determined for each preparation by adding the edema and erythema scores; the formulations will be accordingly classified as non-irritant if PII < 2, irritant if (PII =2-5) and highly irritant if PII=5-8.

Carrageenan-Induced Paw Edema Model: The anti-inflammatory activity of formulated patches was evaluated by the carrageenan-induced rat hind paw edema method of Swingle et al. (1969) [18]. Wistar rats were used after being allowed to acclimatize for 2 weeks. Before the day of administration, rats were fasted overnight but was allowed access to water *ad libitum*. The backsides of rats were shaved 12 h before starting the experiments.

Group I (Control group): Paw edema was induced by injecting 0.1 mL of a 1% w/v homogeneous suspension of carrageenan in double-distilled water. The volume of injected paw was measured immediately (0 h) and at 1, 2, 4, 6, 8, 10, 12 h after injection using a plethysmometer. The amount of paw swelling concerning initial volume was determined from the time to time. It was obtained by subtracting the volume of injected paw at a time '0' from the volume of injected paw at time 't' divided by the volume of injected paw at a time '0'.

Group II (Standard): Treated similarly to the control group except that Aceclofenac patch was applied half an hour before subplantar injection of carrageenan.

Group III (Test groups-3, 4): Treated similarly to the control group except that patches were applied half an hour before subplantar injection of carrageenan. Percent (%) inhibition of edema produced by each patch treated group was calculated against the respective control group using the following formula [16, 17].

$$\% \text{ inhibition of edema} = (1 - Vt / Vc) \times 100$$

Where, Vt = Volume of edema in the test; Vc = Volume of edema in control

TABLE 3: CARRAGEENAN INDUCED PAW EDEMA MODEL

	Group (n=6)	Treatment
Adult Albino Wistar rats	Control	Normal rat chow diet
	Standard group	Aceclofenac patch
	Test group -3	F9 : GA (9 mg) Patch (2 × 2 cm^2)
	Test group -4	F4: GA (9 mg) with piperine (5mg) Patch (2 × 2cm^2)

Stability Study: The stability study was conducted according to ICH guidelines by storing the prepared patch (F4) at 4 ºC, 40 ºC (75 ± 5% RH) and 60ºC kept in the refrigerator, stability chamber and incubator for a period of 6 months. Samples were withdrawn at the end of 6 months and analyzed for physical appearance, drug content, *in-vitro* diffusion studies [19].

RESULTS & DISCUSSION:

Preformulation Studies: It showed white colored crystals GA was poorly soluble in water, buffer solution pH 7.4 while soluble in ethanol, chloroform, DMSO. Interaction of drug with polymers was confirmed by carrying out IR interactions studies. It shows that there are no interactions found between the drug and polymers.

Thickness: % Thickness was observed for all the formulations which were between 0.32 ± 0.004 to 0.33 ± 0.006. A low standard deviation (SD) value in the film thickness measurements ensures uniformity of the patches.

Weight Variation: % Weight variation was observed for all the formulations which were between 1.78 ± 0.015 to 1.79 ± 0.015.

Drug Content Uniformity: % drug content was observed for all the formulations which were between 99.33 ± 0.014% to 99.34 ± 0.016%. The results indicated that the patch preparation was capable of yielding uniform drug content due to the homogenous dispersion of the drug.

***In-vitro* Permeation:** The release of a drug from a transdermal drug delivery system occurs by diffusion. Transport of GA from the polymeric rate controlling membrane (EVA) into *in-vitro* study medium depending upon % of Carbopol Gel base, % of penetrating enhancer menthol, % of vinyl acetate in EVA as well as % of bioavailability enhancer Piperine. The results of release profile indicated that as the % of Carbopol Gel base increased in the patch, the drug release from the patches is decreased (F5>F1, F6>F2, F7>F3, F8>F3).

Concentration of menthol increased from 2% to 5% in the formulation; the in vitro release rate increased (F3> F1, F4> F2, F7> F5, F8> F6).

Hydrophobic nature of EVA polymer retards the drug release, but the percentage of vinyl acetate in the EVA membrane helps in the release of drug from membrane due to pore-forming property. EVA with 19% VA membrane showed greater drug release (F2> F1, F4> F3, F6> F5, F8> F7) as compared to EVA with 9% VA.

0.25%, 0.5% and 1% piperine in the formulations F10, F4, F11 increased bioavailability of GA 30%, 55.44% and 55.77%. However, increasing the concentration of piperine to 1% w/v did not further enhance the permeation of GA.

The enhancement in the permeation of GA (95.5% in F4) in the presence of piperine: methanol (0.5%: 5%) mixture suggested its better performance as compared to that of 0.5 % piperine (50.31% in F12) as well as 5% methanol (40.11% in F9) alone. It is worthy to note that the piperine: methanol (0.5%: 5%) mixture in F4 formulation was significantly more effective for *ex-vivo* analysis of GA. Piperine showed the best role in drug release when added into 0.5% concentration in the formulation, as shown in **Fig. 3** and **Table 4**.

TABLE 3: *IN-VITRO* % CUMULATIVE RELEASE OF GA

Formulations	*In-vitro* drug release of GA					
	0.5 hr	2 hr	4 hr	6 hr	8 hr	10 hr
F1	2.5	11	22	33.44	44	55.88
F2	3.9	15	30	45	60	75
F3	4.2	13.11	26.22	39.33	50.22	65.55
F4	5.1	19	38.22	57.33	76.44	95.55
F5	1.4	7.77	15.55	23.33	31.11	38.89
F6	3.1	11.67	23.33	34.78	46.33	59.11
F7	2.3	9.9	19.66	29.44	39.22	49
F8	4.8	14.11	28.11	43.33	56.22	70.33

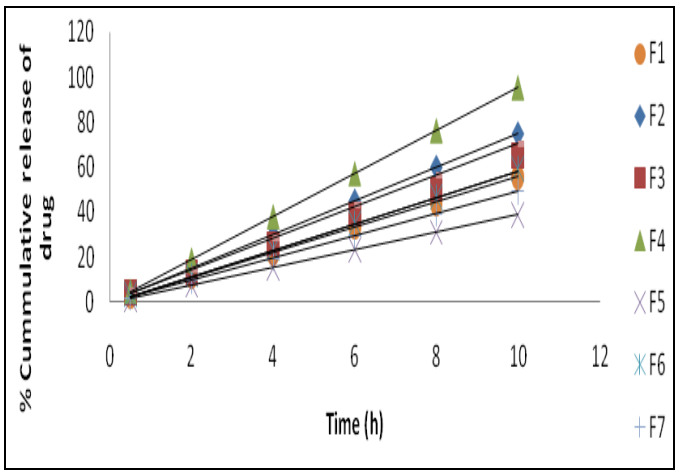

FIG. 2: *IN-VITRO* RELEASE OF GA

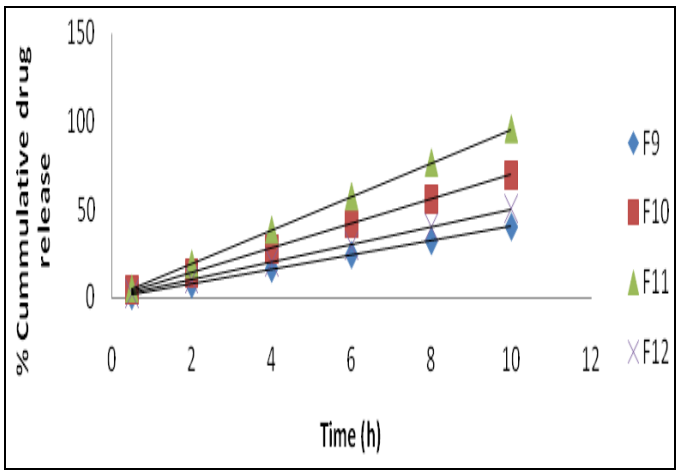

FIG. 3: *IN-VITRO* DRUG RELEASE SHOW BIOENHANCER PROPERTY OF PIPERINE

TABLE 4: *IN-VITRO* DRUG RELEASE SHOW BIOENHANCER PROPERTY OF PIPERINE

Formulations	*In vitro* % cumulative release of GA					
	0.5 hr	2 hr	4 hr	6 hr	8 hr	10 hr
F9	1.2	7.57	16.55	24.33	32.11	40.11
F10	4.7	14.11	27.92	42.33	56.22	70.11
F11	4.9	18.9	37.89	58.11	75.99	94.89
F12	2.6	10.41	19.92	29.93	40.11	50.31

***Ex-vivo* Release of GA:** The prepared F4 transdermal patch of GA was evaluated for *ex-vivo* release pattern. The mechanism of permeation enhancement of menthol could involve its distribution preferentially into the intercellular spaces of stratum corneum and the possible reversible disruption of the intercellular lipid domain [21]. Bioenhancer, piperine increase the bioavailability of the drug by a biphasic mechanism involving partial extraction of stratum corneum (SC) lipid and interaction with SC keratin [22]. *Ex-vivo* study using fresh abdominal skin of goat as permeability membrane confirms the release of GA in the patch as controlled delivery over 10 h as 91.58%.

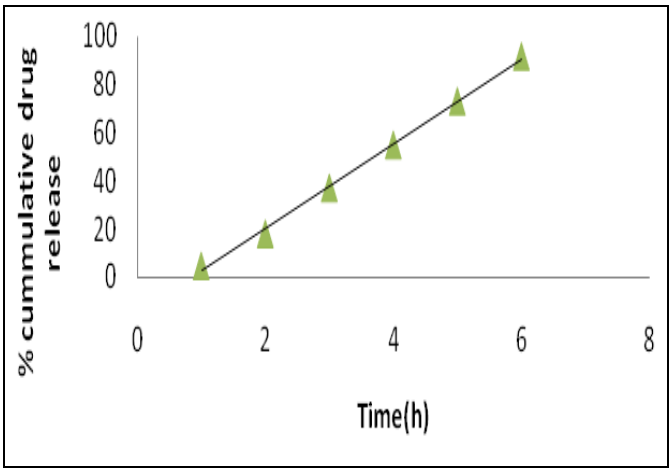

FIG. 4: *EX-VIVO* RELEASE OF GA

TABLE 5: *EX-VIVO* RELEASE OF GA

Formulations	*Ex-vitro* % cumulative release of GA					
	0.5 hr	2 hr	4 hr	6 hr	8 hr	10 hr
F4	4.93	18.23	37.17	56.58	73.01	91.58

Biological Evaluation:

Skin Irritation Test: According to Draize et al. the tested transdermal patch was considered to be negative (non-irritant)[PII< 2].Statistical analysis using Two way ANOVA test at $p < 0.05$ showed that, compared to the control, the formalin solution was found to be significantly irritant [p = 2]. The irritation indices proved the no irritancy of the drug or any of patch components and showed that the innovated patches are safe to be applied to the skin for the intended period.

TABLE 6: SKIN IRRITANCY OF TRANSDERMAL PATCHES

Group (n=6)	Erythema scale	Edema scale	PII
A. Std (Aceclofenac Patch)	0 (None)	0 (None)	< 2 (Non irritant)
B.Disease Control (Formalin)	2 (well defined)	2 (well defined)	2 (Irritant)
C. Test (GA with piperine) patch	0 (None)	0 (None)	< 2 (Non irritant)

Carrageenan-Induced Paw Edema Model: The paw of rats is very sensitive to carrageenan when it's injected in the subplantar region of the left hind paw it causes swelling, redness, and edema of the paw. GA transdermal patches have been proved to decrease the swelling of the injected paw according to the equation.

From rat paw edema volume in **Table 7,** we noticed that the control group showed a continues increase in paw swelling edema till 4 h while both the std. and test groups showed percent expanding lower than that of the control group. For Test group-4, there was a decrease in the percentage of swelling was better than std. group after 1 h. While for test groups 3 showed a reasonably gradual decrease in the percentage swelling. The paw volume nearly returned to normal at 12 h.

TABLE 7: CARRAGEENAN INDUCED RAT PAW EDEMA VOLUME (ml)

Time (hr)	Disease control Group (Vc)	Standard Group A + P (Vt)	Test Group-3 GA (Vt)	Test Group -4 GA + P (Vt)
0	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
1	0.58 ± 0.04	0.31 ± 0.04	0.41 ± 0.04	0.28 ± 0.04
2	1.11 ± 0.04	0.51 ± 0.04	0.76 ± 0.05	0.41 ± 0.04
4	1.48 ± 0.04	0.41 ± 0.04	0.96 ± 0.05	0.38 ± 0.04
6	1.38 ± 0.04	0.28 ± 0.04	0.90 ± 0.06	0.26 ± 0.05
8	1.28 ± 0.04	0.18 ± 0.04	0.81 ± 0.04	0.21 ± 0.05
10	0.88 ± 0.04	0.11 ± 0.04	0.55 ± 0.05	0.11 ± 0.02
12	0.15 ± 0.05	0	0.09 ± 0.02	0

Each Group n=6 rats taken

TABLE 8: ANTI INFLAMMATORY ACTION OF TRANSDERMAL PATCHES

Time (hr)	% Inhibition of edema		
	Standard Group A + P	Test Group-4 GA+P	Test Group-3 GA
0	0	0	0
1	45.71	51.43	28.57
2	53.73	62.68	31.34
4	71.91	74.16	34.83
6	79.52	80.72	34.93
8	85.71	87.01	36.36
10	86.79	89.62	37.73
12	100	100	38.88

Statistical analysis of the data using Two-way ANOVA showed a significant difference ($p<0.05$) between the std. group and all other groups regarding % inhibition of edema. Moreover, there was a significant difference ($p<0.05$) between three formulations [23].

Stability Study: To determine the change in physicochemical parameter and *in-vitro* release profile on storage, stability study was carried out as per ICH Q1A (R2) guidelines. The physicochemical parameter and *in-vitro* release of the optimized formulation were not significantly

changed on storage. The result indicates that the formulation was stable on the required storage condition.

CONCLUSION: *In-vivo* anti-inflammatory activity of GA with piperine patch was much higher compared to Aceclofenac patch and GA patch of equivalent concentration. From *in-vivo* data has proved the feasibility of controlled transdermal delivery of GA in adequate quantity into the circulation.

ACKNOWLEDGEMENT: The authors thank Faculty of Pharmacy, DDU, Nadiad, Gujarat, India for providing required facilities to carry out this research work.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Rajpal V: Standardization of botanicals.vol.-1. 2nd Ed. New Delhi: Business Horizons 2011: 115-139.
2. Li YJ and Bi KS: Study on the therapeutic material basis of traditional Chinese medicinal preparation suanzaoren decoction. Chem Pharm Bull 2006; 54(6): 847-51.
3. Chung-Yi W: Glycyrrhizic acid and 18β-glycyrrhetinic acid modulate lipopolysaccharide-induced inflammatory response. J Agric Food Chem 2011; 59(14): 726-33.
4. Li S: A novel transdermal formulation of 18β-glycyrrhetic acid with lysine for improving bioavailability and efficacy. Skin Pharmacol Physiol 2012; 25(5): 257-68.
5. Cosmetic Ingredient Review Expert Panel. Final report on the safety assessment of Glycyrrhetinic Acid. Int J Toxicol 2007; 26(2): 79-112.
6. Harle UN: Emerging challenge of herb-drug interaction. Indian J Pharm. Educ 2005; 39(2).
7. Kaushal N: Influence of piperine on transcutaneous permeation of repaglinide in rats and on tight junction proteins in HaCaT Cells: Unveiling the Mechanisms for Enhanced Permeation.Scientia Pharmaceutica 2009; 77: 877-897.
8. Goswami DS: Permeation Enhancer for TDDS from Natural and Synthetic Sources: A Review. Journal of Biomedical and Pharmaceutical Research 2013; 2(1): 19-29.
9. Singh VK: Formulation and evaluation of topical gel of aceclofenac containing piperine. Indo American Journal of Pharm Research 2013; 3(7): 5266-5280.
10. Patel: Formulation and evaluation of transdermal patch of Aceclofenac International Journal of Drug Delivery 2009; 1: 41-51.
11. Yadav S: Formulation and evaluation of transdermal patch for antirheumatic ayurvedic medicine using different polymer compositions: *in-vitro*. Journal of Global Trends in Pharmaceutical Sciences 2013; 4(1): 999-1006.
12. Gonzalez N and Sumano H: Design of two liquid ibuprofen-poloxamer- limonene or menthol preparations for dermal administration. Drug Delivery 2007; 14: 287-293.
13. Naseera: Formulation, optimization and evaluation of matrix type of transdermal system of simvastatin using permeation enhancers. Int J Curr Pharm Res 2012; 4(2): 79-87.
14. Draize J, Woodward G and Calvery H: Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944; 82: 377-79.
15. Mutalki S and Udupa N: Pharmaceutical evaluation of membrane moderated transdermal system of glipizide. Clin Exp Pharmacol Physiol 2006; 33: 17-27.
16. Winter CA, Risley EA and Nuss GW: Carrageenin-induced edema in the hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Biol Med 1962; 11: 544-47.
17. Panchaxari DM: Design and characterization of diclofenac diethylamine transdermal patch using silicone and acrylic adhesives combination. DARU Journal of Pharmaceutical Sciences 2013; 21(6): 1-14.
18. Swingle KF, Grant TJ, Jacques LW and Kvam DC: Interaction of anti-inflammatory drugs in carrageenan-induced foot edema of the rat. J Pharmacol Exp Ther 1969; 172: 423 - 25.
19. Jayprakash S: Formulation, *in-vitro* and *in-vivo* characterization of Transdermal patches of etoricoxib. International Journal in a Review of Life Science 2012; 2(1): 17-23.
20. Pharmaceutical statistics: practical and clinical applications, Bolton S. Ed., Taylor & Francis, New York, 2003.
21. Kunta JR: Effect of menthol and related terpenes on the percutaneous absorption of propranolol across excised hairless mouse skin. J Pharm Sci 1997; 86(12): 1369-73.

22. Shah K: Transdermal delivery of aceclofenac: effect of piperine and its Mechanism of action. International Journal of Pharma and Biosciences 2011; 2(3): 10-18.

23. Pharmaceutical statistics: practical and clinical applications, Bolton S. Ed., Taylor & Francis, New York, 2003.

How to cite this article:
Patel PM and Bhaskar V: *In-vitro, ex-vivo* skin permeation and biological evaluation of 18-β-glycyrrhitic acid transdermal patches. Int J Pharmacognosy 2015; 2(8): 400-08. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(8).400-08.