FORMULATION, OPTIMIZATION, AND EVALUATION OF TRANSDERMAL PATCHES OF ECLIPTA ALBA LEAF EXTRACT

Gireesh Tripathi* and Narendra Lariya

RKDF University Bhopal Madhya Pradesh

Email: informa.pharmaceutics@gmail.com

* Corresponding Author

Gireesh Tripathi

Research Scholar

RKDF University Bhopal Madhya Pradesh

Email: informa.pharmaceutics@gmail.com

ABSTRACT

The purpose of this research was to develop a matrix-type transdermal therapeutic system containing Eclipta Alba leaf extract with different ratios of hydrophilic polymeric systems by the solvent evaporation technique by using 30% (w/w) of PEG 400 LR to the dry polymer weight, incorporated as a plasticizer. A methanolic extract of Eclipta Alba was produced and analyzed using the Maceration process. Different concentrations of oleic acid and isopropyl myristate were used to enhance the transdermal permeation of Eclipta Alba leaf extract. The physicochemical compatibility of the drug and the polymers studied by infrared spectroscopy suggested the absence of any incompatibility. The developed transdermal patches were assessed for their physiochemical properties, including physical appearance, uniformity of weight, thickness, folding endurance, and pH. All transdermal patches exhibited a variable range of thickness from 0.114±0.01 to 0.145±0.01 mm and weight from 7.00±0.75mg to 7.98±0.70mg. The content uniformity ranged from 9.9±0.29 to 10.1±0.43µg/cm2. The range of folding endurance was found to be from 80 to 205. The developed F5 formulation patch demonstrated a 93.02% in vitro drug release at 10 hours. All prepared formulations indicated good physical stability. E8 formulation prepared with 3% HPMC K15M polymer containing permeation enhancer showed the best *in-vitro* skin permeation through Wistar albino rat skin compared to all other

formulations. Formulation E8 showed the highest flux among all the formulations and 1.46-fold enhancements in drug permeation. These results indicate that the formulation prepared with 3% HPMC K15M polymer containing 10% oleic acid and 10% isopropyl myristate gives better Eclipta Alba leaf extract penetration through rat skin.

KEY WORDS: Eclipta Alba, transdermal patch, anti-inflammatory, maceration, sustained release.

INTRODUCTION

Transdermal drug administration generally refers to the topical application of agents to healthy, intact skin for localized treatment of tissues underlying the skin or for systemic therapy. For transdermal products, the goal of dosage design 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 (1). Transdermal drug deliveryhas many advantages over the oral route of administration, such as improving patient compliance in long-term therapy, bypassing first-pass metabolism, sustaining drug delivery, maintaining a constant and prolonged drug level in plasma, minimizinginter- and intra-patient variability, and making it possible to interrupt or terminate treatment when necessary (2-3).

Eclipta alba is a herb growing in damp, moist puddles distributed in the tropical and subtropical regions of the world (4). The main active principles include coumestans like wedelolactone, desmethylwedelolactone (5), furanocoumarins, and oleanane and taraxastane glycosides (6). Various biological activities are possessed by E. alba, such as memory disorders treatment, general tonic, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity, and skin disorders (7-8). Wedelolactone is a leading active compound of Eclipta alba and is well-known for inhibiting antioxidant, anti-inflammatory, and 5-lipoxygenase activities.

The objectives of the present investigation were to (a) prepare and characterize Eclipta Alba leaf extract, (b) formulate the herbal transdermal patches using hydrophilic polymers, (c) optimize transdermal patch formulation using 3^2 full factorial design, and (d) study the *in-vitro* diffusion behavior of prepared transdermal patch formulations in the presence and absence of penetration enhancer.

MATERIALS AND METHODS

Materials

Dried leaves of Eclipta alba were procured from Shobhasavi AYURVEDICS and agros, Bhopal, India. The plant was identified in the Department of Botany, Shri Krishna University, Chhatarpur, Madhya Pradesh, India. HPMC was purchased from SD fine Ltd., Indore, India. Oleic acid (OA), polyethylene glycol (PEG) 400 LR, and Di-n-butyl phthalate (DBP) were procured from Sigma Chemicals Ltd., Mumbai, India. Other materials used in the study (chloroform, methanol, dichloromethane, glycerol, potassium dihydrogen phosphate, etc.) were of analytical grade. Milli-Q water was used throughout the study.

Preparation of Eclipta Alba leaf extract

300 g each of powdered material was extracted with 600mL of methanol by cold maceration process to obtain the methanolic extract. The prepared extract was concentrated in a vacuum to dryness and stored at room temperature under dark conditions for further processing (9).

Phytochemical tests

The prepared leaf extracts were evaluated for their chemical constituents as the previously reported methods described. The plant extract was subjected to TLC, and the developed spots were observed for terpenoids, alkaloids, and flavonoids, respectively (10).

Investigation of physicochemical compatibility of extract and polymer

The physicochemical compatibility between Eclipta Alba leaf extract and polymers used in the patch was studied using Fourier transform infrared (FTIR- 8300, Shimadzu Co., Kyoto, Japan) spectroscopy. The infrared (IR) spectra were recorded using an FTIR by the KBr pellet method, and spectra were recorded in the wavelengthregion between 4,000 and 400 cm–1. The spectra obtained for extract, polymers, and physical mixtures of the extract with polymers were compared (11).

Preparation of transdermal patch

Transdermal patches containing Eclipta Alba leaf extract were prepared by solvent evaporation in cylindrical glass molds with both sides open (12). The backing membrane was cast by pouring a 2% (m/V) polyvinyl alcohol (PVA) solution, followed by drying at 60°C for six h. The extract reservoir was prepared by dissolvingthe HPMC polymer in Milli-Q water. PEG 400 LR 30% (w/w of dry polymer composition) was used as a plasticizer (13). Ten mg of the extract was added into the

homogeneous dispersion with slow stirring on a magnetic stirrer. The uniform dispersion was cast on a PVA backing membrane and dried at room temperature (Table 1). The films were stored between sheets of wax paper in a desiccator (14).

Table 1. Composition of transdermal patches

Formulation code	PVA (backing membrane)	HPMC K4	HPMC K15	Oleic acid	PEG 400 LR	Water
F1	2%	2.0%	-	0.2 mL	30 % w/v of total ploymer composition	q.s.
F2	2%	3.0%	-	0.2 mL		q.s.
F3	2%	4.0%	-	0.2 mL		q.s.
F4	2%	-	2.0%	0.2 mL		q.s.
F5	2%	-	3.0%	0.2 mL		q.s.
F6	2%	-	4.0%	0.2 mL		q.s.

Note: * 30 % w/w of dibutyl phthalate to the polymer weight, incorporated as plasticizer.

Physicochemical characterization of transdermal patch

Thickness

The thickness of patches was measured at three different places using a micrometer (India Tools & Instruments Co., Mumbai), and mean values were calculated (15).

Weight variation

The weight variation of patches was measured by individually weighing randomly selected patches. Such determinations were carried out for each formulation (16).

Drug content

Patches of a specified area (1 cm^2) were dissolved in 5 mL of Phosphate buffer pH 7.4, and the volume was made up to 10 mL with the same buffer. A blank was prepared using a drug-free patch treated similarly. The solutions were filtered through a 0.45 µm membrane, diluted suitably, and absorbance was read at 382nm in a double-beam UV-Visible spectrophotometer (UV-1800 Shimadzu, Japan).

Folding endurance

Folding endurance was determined by repeatedly folding one film in the same place until it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value for folding endurance.

Tensile strength

In order to determine the elongation as tensile strength, the polymeric patch was pulled using a pulley system; weights were gradually added to the pan to increase the pulling force till the patch was broken. The elongation i.e., the distance traveled by the pointer before the break of the patch, was noted with the help of a magnifying glass on the graph paper, and tensile strength was calculated as $kg \cdot cm^{-2}$.

***In-vitro* skin permeation studies**

In-vitro skin permeation studies were performed using a Diffusion Cell Apparatus with a receptor compartment (17). Excised rat abdominal skin (Wistar albino) was mounted between the donor and receptor compartments of the diffusion cell. The formulated patches were placed over the skin and covered with paraffin film/or a backing layer. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The solution in the receptor compartment was constantly stirred using magnetic beads at 50 rpm; the temperature was maintained at 37±0.5°C. Samples were withdrawn at different time intervals and analyzed for drug content spectrophotometrically (18). The receptor phase was replenished with an equal volume of phosphate buffer pH 7.4 at each sample withdrawal. The cumulativepercentages of drug permeated per square centimeter of patches were plotted against time (19).

Full factorial design

A 3^2 randomized full factorial design was used in the present study. In this design, two factors were evaluated, each at three levels and experimental trials was performedat all nine possible combinations. The amount of isopropyl myristate (X1) and oleic acid (X2) were selected as independent variables. Drug release at ten h was selected as the dependent variable (20).

Stability study of optimized formulation

A stability study was carried out for optimized patch formulation at 40°C temperature in a humidity chamber having 75% RH for three months. After three months, samples were withdrawn and evaluated for physicochemical properties and *in-vitro* diffusion studies.

RESULTS AND DISCUSSION

Phytochemical analysis of Eclipta alba leaf extract

The extractive values of methanolic extract were good and exhibited the required solubility in the solvents for its biological evaluation. The plant extracts of Eclipta

alba have been extracted by maceration as per the British Pharmaceutical codex. The phytochemical studies revealed the presence of alkaloids, saponins, tannins, phenolic compounds, flavonoids, and sterols (Table 2).

Table 2. Phytochemical characterization of Eclipta alba leaf extract

S.No.	Chemical Constituent	Extract
	Alkaloid	+
	Saponin	+
	Tannins	+
	Phenolic compounds	+
	Flavonoids	+
	Sterol	+
	Glycosides	+
	Amino acids	-
	Reducing sugar	-
.	Terpenoids	-

(+) Present (-) Absent

Investigation of physicochemical compatibility of extract and polymer

FTIR techniques have been used here to study the physical and chemical interaction between the extract and excipients used. The study observed no changes in these prominent peaks in IR spectra of a mixture of drug and polymers, showing no physical interactions because some bond formation between extract and polymerexcipients are compatible with extract within the formulation.

Physicochemical characterization of transdermal patch

The thickness ranged between 0.114±0.01 to 0.145±0.01, indicating that they were uniform. The weights ranged between 7.00±0.75mg and 7.98±0.70mg, which indicates that different batches' patch weights were relatively similar. Good drug content uniformity among the batches was observed with all formulations, ranging from 9.9±0.29 to 10.1±0.43μg/cm^2. The results indicate that the process employed to prepare patches in this study could produce patches with uniform drug content and minimal patch variability. Folding endurance and Tensile strength test results indicated that the patches would not break and would maintain their integrity with general skin folding when applied (Table 3).

Table 3. Evaluation of transdermal patch formulations of Eclipta alba leaf extract

Formulation code	Thickness uniformity (mm)	Weight variation test (mg)	Drug content (μg/cm^2)	Folding endurance	Tensile strength (kg/cm^2)
F1	0.116 ± 0.011	7.03 ± 0.73	9.9 ± 0.42	180 ± 3	2.0 ± 0.5
F2	0.114 ± 0.01	7.00 ± 0.75	9.8 ± 0.75	205 ± 4	2.5 ± 0.25
F3	0.118 ± 0.005	7.12 ± 0.45	9.5 ± 0.44	80 ± 10	3.0 ± 0.75
F4	0.140 ± 0.015	7.03 ± 0.95	10.1 ± 0.43	135 ± 4	2.0 ± 0.25
F5	0.126 ± 0.01	7.24 ± 0.95	9.9 ± 0.52	196 ± 2	3.5 ± 1.0
F6	0.145 ± 0.01	7.98 ± 0.70	9.8 ± 0.29	92 ± 5	3.5 ± 0.5

*** mean ± SD (n = 3).**

In-vitro skin permeation

The *in-vitro* release profile is an important tool that predicts a drug's behavior in-vivo. The results of *in-vitro* skin permeation studies of extract from transdermal patches are shown in Figure 1. The present study used hydrophilic polymers (HPMC K4M and HPMC K15M) to prepare patches. Formulation F5 exhibited the greatest, 93.02%, drug release value, while formulation F1 exhibited the lowest, 73.65%, drug release value. The cumulative amount of drug released from formulations containing hydrophilic polymer HPMC K4M released the drug faster than HPMC K15M. The cumulative amount of drug released from formulations F1, F2, and F4 was much higher than other formulations. The transdermal drug delivery system F6 (4% HPMC K 15 M) showed drug release (88.16%) and lasted only 8h. However, the transdermal drug delivery system F5 (3% HPMC K15) showed the highest prolonged drug release successfully for 10h (93.02%). F5 achieved a high cumulative drug permeation at the end of 10 h. Based on physicochemical and *in-vitro* release experiments, F5 was chosen for further studies.

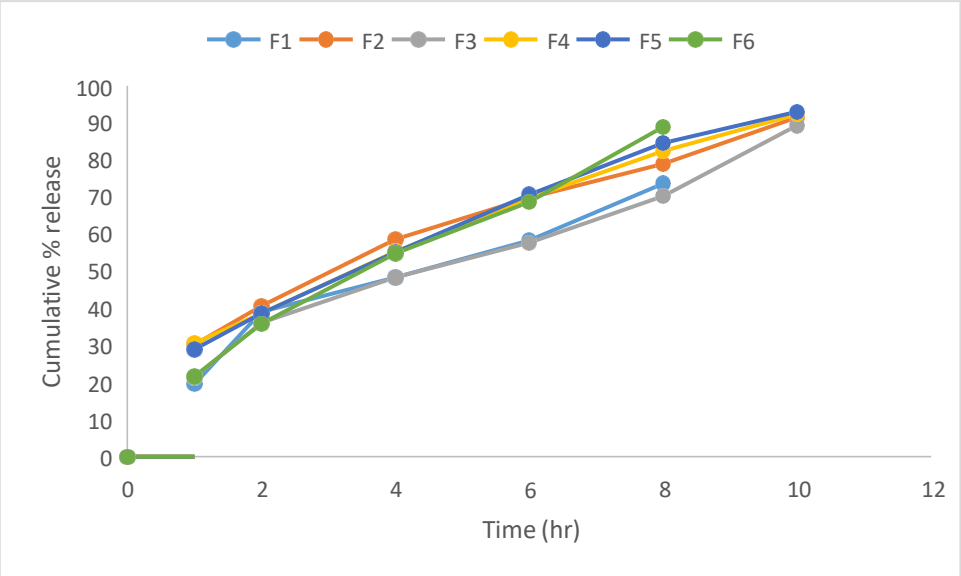

Figure 1. Release profiles of extract from patches containing different concentrations of HPMC K4M and HPMC K15M

Full factorial design

The cumulative percentage of drug permeated through the rat epidermis from the patch containing different concentrations of penetration enhancer was studied. An increase in the concentration of oleic acid leads to an increase in Q10h because the coefficient b1 bears a positive sign. Increasing the concentration of oleic acid from 5 to 10%, the Q10h value increased from 81.13% to 88.14%. Increasing the concentration of isopropyl myristate increases Q10h because the coefficient b2 bears apositive sign. When the concentration of isopropyl myristate increased from 5 to 10%,the Q10h value increased from 84.36% to 90.31%.

Here the coefficient of interaction terms showed a negative value. The interaction term indicated that Q10h was not significantly affected by the interaction of two penetration enhancers. This indicates that changing two factors at a time had no effect on Q10h. The maximum amount (Q10h) of extract that permeated during the 10 hr of the study was 93.06% from formulation E8. The flux was calculated by dividing the cumulative amount of drug which permeated per cm^2 of the skin with time. Thus the corresponding flux of extract was 292.03 $\mu g \cdot cm^{-2}$ h^{-1} from formulation E8. A marked effect of penetration enhancers on extract permeation was observed when they were incorporated into the patch in varying concentrations. The cumulative percentage of extract that permeated over 10 h increased, ranging from 67.91 to 93.06% for patches.

The corresponding flux values ranged from 208.50 to 292.03 $\mu g \cdot cm^{-2}\ h^{-1}$. Formulation E8 shows the highest flux among all the formulations. This result indicated that the formulation containing 10% oleic acid with 10% isopropyl myristate gave better penetration of extract through rat skin (Table 4 and Table 5).

Table 4. 3^2 full factorial design layouts for transdermal patches

Batch No.	X1	X2	Q10h release (%)	Flux (J) ($\mu g \cdot cm^{-2} \cdot h^{-1}$)	Permeability co efficient (K) ($cm \cdot h^{-1}$)	Enhancement ratio (ER)
E 1	-1	-1	68.45	218.50	4.18	1.04
E 2	1	1	88.01	264.12	5.25	1.32
E 3	0	1	88.78	269.74	5.36	1.34
E 4	1	-1	69.48	215.20	4.24	1.06
E 5	1	0	83.12	255.60	5.06	1.26
E 6	0	0	82.65	252.27	5.04	1.25
E 7	0	-1	69.18	218.50	4.18	1.04
E 8	-1	0	96.75	294.03	5.86	1.46
E 9	-1	1	93.32	282.63	5.64	1.41

Table 5: Translation of coded levels in actual units

Variables level	Low (-1)	Medium (0)	High (+1)
Amount of isopropyl myristate (% W/W of drug) X1	0	5	10
Amount of oleic acid (% W/W of drug) X2	0	10	15

Kinetic modeling of drug release

The cumulative amount of drug permeated per square centimeter of patches (E1 to E9) through rat skin plotted against time was fitted to zero, first, and Higuchi kinetic models. The release profile of H followed mixed zero-order and first-order kinetics in different formulations. The release profile of patches E1 to E9 was evaluated per Higuchi's equation. The release profile of the optimized formulation E8 (r^2 = 0.995 for

Higuchi) indicated that a diffusion mechanism governed the permeation of the drug from the patches.

Stability studies

In order to determine the change in physicochemical parameters and *in-vitro* release profile on storage, a stability study was carried out. The physicochemical parameters of the optimized formulation were not significantly changed on storage. The *in-vitro* release profile before and after storage was also very similar. The result indicates that the formulation was stable under the required storage conditions.

CONCLUSION

The present work has achieved the objectives of formulation of a transdermal patch of Eclipta Alba leaf extract by using different polymers. The diffusion kinetics confirms that the formulation followed zero order, non-fiction diffusion model. All formulations also showed good physicochemical properties like thickness, weight variation, drug content, folding endurance, and tensile strength. The in-vitro release data showed that polymer types and concentrations affected drug release from the patch formulation. The effect of penetration enhancers like oleic acid and isopropyl myristate indicated that as the concentration of penetration enhancers increased drug permeation was increased.

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REFERENCES

1. Jahan R, Al-Nahain A, Majumder S, Rahmatullah M. Ethnopharmacological significance of Eclipta alba (L.) hassk. (Asteraceae). Int Sch Res Notices [Internet]. 2014;2014:385969. Available from: http://dx.doi.org/10.1155/2014/385969 PMCID: PMC4897414
2. Chaudhary H, Dhuna V, Singh J, Kamboj SS, Seshadri S. Evaluation of hydro-alcoholic extract of Eclipta alba for its anticancer potential: an in vitro study. J Ethnopharmacol [Internet]. 2011;136(2):363–367. Available from: http://dx.doi.org/10.1016/j.jep.2011.04.066 PMID: 21575697
3. Yadav NK, Arya RK, Dev K, Sharma C, Hossain Z, Meena S, Arya KR, Gayen JR, Datta D, Singh RK. Alcoholic extract of Eclipta alba shows in vitro antioxidant and anticancer activity without exhibiting toxicological effects. Oxid

Med Cell Longev [Internet]. 2017;2017:9094641. Available from: http://dx.doi.org/10.1155/2017/9094641 PMCID: PMC5307245

4. Pandey PK, Parashar AK. Formulation, characterization and evaluation of liposomal hydrogel for the treatment of antibiotic resistant Propionibacterium acne. Curr Res Pharm Sci [Internet]. 2021;11(2):65–71. Available from: http://dx.doi.org/10.24092/crps.2021.110204
5. Thakur VD, Mengi SA. Neuropharmacological profile of Eclipta alba (Linn.) Hassk. J Ethnopharmacol [Internet]. 2005;102(1):23–31. Available from: http://dx.doi.org/10.1016/j.jep.2005.05.037 PMID: 16054316
6. Govindarajan M, Karuppannan P. Mosquito larvicidal and ovicidal properties of Eclipta alba (L.) Hassk (Asteraceae) against chikungunya vector, Aedes aegypti (Linn.) (Diptera: Culicidae). Asian Pac J Trop Med [Internet]. 2011;4(1):24–28. Available from: http://dx.doi.org/10.1016/S1995-7645(11)60026-6 PMID: 21771410
7. Parashar AK, Patel P, Gupta AK, Jain NK, Kurmi BD. Synthesis, characterization andin vivo evaluation of PEGylated PPI dendrimer for safe and prolonged delivery of insulin. Drug Deliv Lett [Internet]. 2019;9(3):248–63. Available from: http://dx.doi.org/10.2174/2210303109666190401231920
8. Shaikh MF, Sancheti J, Sathaye S. Effect of Eclipta alba on acute seizure models: A GABAA-mediated effect. Indian J Pharm Sci [Internet]. 2013;75(3):380–384. Available from: http://dx.doi.org/10.4103/0250-474X.117432 PMCID: PMC3783761
9. Diogo LC, Fernandes RS, Marcussi S, Menaldo DL, Roberto PG, Matrangulo PVF, Pereira PS, França SC, Giuliatti S, Soares AM, Lourenço MV. Inhibition of snake venoms and phospholipases A(2) by extracts from native and genetically modified Eclipta alba: isolation of active coumestans. Basic Clin Pharmacol Toxicol [Internet]. 2009;104(4):293–299. Available from: http://dx.doi.org/10.1111/j.1742-7843.2008.00350.x PMID: 19320636
10. Lee MK, Ha NR, Yang H, Sung SH, Kim GH, Kim YC. Antiproliferative activity of triterpenoids from Eclipta prostrata on hepatic stellate cells. Phytomedicine [Internet]. 2008;15(9):775–780. Available from: http://dx.doi.org/10.1016/j.phymed.2007.10.004 PMID: 18061418
11. Abdul Rasool BK, Mohammed AA, Salem YY. The optimization of a dimenhydrinate transdermal patch formulation based on the quantitative analysis

of in vitro release data by DDSolver through skin penetration studies. Sci Pharm [Internet]. 2021;89(3):33. Available from: http://dx.doi.org/10.3390/scipharm89030033

12. Cherukuri S, Batchu UR, Mandava K, Cherukuri V, Ganapuram KR. Formulation and evaluation of transdermal drug delivery of topiramate. Int J Pharm Investig [Internet]. 2017;7(1):10–17. Available from: http://dx.doi.org/10.4103/jphi.JPHI_35_16 PMCID: PMC5370344
13. Patel RP, Gaiakwad DR, Patel NA. Formulation, optimization, and evaluation of a transdermal patch of heparin sodium. Drug Discov Ther [Internet]. 2014;8(4):185–193. Available from: http://dx.doi.org/10.5582/ddt.2014.01030 PMID: 25262597
14. Samiullah, Jan SU, Gul R, Jalaludin S, Asmathullah. Formulation and evaluation of transdermal patches of pseudoephedrine hcl. Int J Appl Pharm [Internet]. 2020;121–127. Available from: http://dx.doi.org/10.22159/ijap.2020v12i3.37080
15. Akram R, Ahmad M, Abrar A, Sarfraz RM, Mahmood A. Formulation design and development of matrix diffusion controlled transdermal drug delivery of glimepiride. Drug Des Devel Ther [Internet]. 2018;12:349–364. Available from: http://dx.doi.org/10.2147/dddt.s147082
16. Patel NA, Patel NJ, Patel RP. Design and evaluation of transdermal drug delivery system for curcumin as an anti-inflammatory drug. Drug Dev Ind Pharm [Internet]. 2009;35(2):234–242. Available from: http://dx.doi.org/10.1080/03639040802266782 PMID: 18785045
17. Parashar AK. Synthesis and characterization of temozolomide loaded theranostic quantum dots for the treatment of brain glioma. J Med Pharm Allied Sci [Internet]. 2021;10(3):2778–82. Available from: http://dx.doi.org/10.22270/jmpas.v10i3.1073
18. Saoji SD, Atram SC, Dhore PW, Deole PS, Raut NA, Dave VS. Influence of the component excipients on the quality and functionality of a transdermal film formulation. AAPS PharmSciTech [Internet]. 2015;16(6):1344–1356. Available from: http://dx.doi.org/10.1208/s12249-015-0322-0 PMCID: PMC4666255
19. Parashar AK. Synthesis and characterization of ligand anchored poly propyleneiminedendrimers for the treatment of brain glioma. J Med Pharm Allied Sci [Internet]. 2021;10(3):2784–2789. Available from: http://dx.doi.org/10.22270/jmpas.v10i3.1084

20. Yamsani VV, Mudulaghar MK, Afreen S, Wajid S, Ravula SK, Babelghaith SD. Formulation design and in vitro ex vivo evaluation of transdermal patches of Cinnarizine. Pak J Pharm Sci. 2017;30(6):2075–2083. PMID: 29175776