Chapter 9

Design, characterization and evaluation of transdermal patches of rebamipide in rodent model of PD

9.1 Introduction

Transdermal delivery is one of the advanced approaches for drug administration and currently also used for the treatment of neurodegenerative disorders, such as PD. Rebamipide is poorly absorbable and its plasma protein binding is high (98.4-98.6%) (Naito and Yoshikawa, 2010). As stated earlier, after oral administration once, only 71% and 41% radioactivity of ^{14}C-labeled rebamipide was observed in the brain of rats when compared to blood and plasma levels (Shioya et al., 1989). The low permeability and low aqueous solubility may be responsible for it because according to the USFDA (United States Food and Drug Administration), rebamipide is classified as BCS (Biopharmaceutics Classification System) Class-IV agent (Pradhan et al., 2015; Stojančević et al., 2013). Solubility is the most important rate-limiting parameter for orally administered drugs to achieve the required bioavailability and elicit pharmacological response (Savjani et al., 2012). Lipid-made cell surface, such as blood brain barrier is penetrated by lipophilic drugs rapidly compared to hydrophilic drugs (Alavijeh et al., 2005), whereas hydrophilic drugs are absorbed faster through the gastrointestinal membrane (Ashford, 2017). The absorption is found to be slower for drugs having poor water solubility which is responsible for their inadequate bioavailability (Savjani et al., 2012). Rebamipide is highly lipophilic and hydrophobic drug (Ngo et al., 2017; Pradhan et al., 2015), due to which it may not be well absorbed over gastrointestinal membrane through passive diffusion (Ashford, 2017; Huang et al., 2008), but is able to permeate the blood brain barrier (Fukui et al., 2017). This is the reason that the drug has low oral bioavailability (4.8 ± 1.4%) (Shin et al., 2004) and it is found to be effective in PD, but only in high doses (80 mg/kg oral. twice a day), as concluded in Chapter 5 (Mishra and Krishnamurthy,

2019). Therefore, the prime objective of the present study is to decrease the required effective dose and frequency of rebamipide to act against PD model in rats.

Various formulations to increase the oral bioavailability and aqueous solubility of rebamipide have already been tried, such as mucoadhesive tablets (Gudas et al., 2016), solid dispersion tablets (Pradhan et al., 2015), gastro-retentive tablets (Ha et al., 2017), micro emulsions (Kim et al., 2017) and nano-crystal tablets (Guo et al., 2015). Among the newer methodologies for administration of drugs, is the use of transdermal approaches. Transdermal drug delivery has advantages over other routes because transdermal patches deliver drugs directly into the circulatory system, bypassing the gastrointestinal system. Therefore, this route may require low dose to achieve the desired pharmacological effect (Isaac and Holvey, 2012). The patches of rivastigmine (9.5 mg/ 24 hours) are reported to provide comparable drug exposure to capsules (12 mg/day) (Lefevre et al., 2008). Transdermal patches have been designed as the therapeutics of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease (Isaac and Holvey, 2012). Currently, several drugs are available as transdermal patch systems for neurological and other disorders in adults, which include rotigotine, lidocaine, rivastigmine, diclofenac and capsaicin (Farlow and Somogyi, 2011). Rotigotine (DA agonist), highly lipid soluble and poorly water-soluble drug is available in market as transdermal patches which are applied only once a day in human for symptomatic treatment of PD (Waters, 2013). Topical rotigotine is also reported to improve locomotor activity up to 48h against MPTP-lesioned marmoset. However, rotigotine when given through i.p. and oral route once a day, it could improve locomotor activity in the same model only up to 2h

(Löschmann et al., 1989). Therefore, transdermal route is helpful in reducing the dose frequency.

Drugs having poor oral absorption, short half-life and low tolerability of the oral formulation may be administered through transdermal delivery (Farlow and Somogyi, 2011). There are some ideal properties of drug candidate for transdermal delivery. Rebamipide also fulfills the criteria, such as log P (partition coefficient between water and 1-octanol) should range from 1-4 (rebamipide = 2.4) (Chandrashekar and Rani, 2008; Obae et al., 2017); molecular weight should be < 600 Dalton (rebamipide = 370.786 Dalton) (Shabbir et al., 2014); lipophilicity, an important parameter for transdermal administration because lipid solubility is responsible for systemic absorption of topically-administered drugs (Prausnitz and Langer, 2008); half-life should be 10h or less (rebamipide = 7.48 ± 0.92h) (Chandrashekar and Rani, 2008; Cooper et al., 2014); low oral bioavailability (rebamipide = 4.8 ± 1.4%) (Chandrashekar and Rani, 2008; Shin et al., 2004); hydrophobic drug with aqueous solubility > 1mg/mL (water-solubility of rebamipide = 0.00766 mg/mL) (Naik et al., 2000). Having these properties, rebamipide is an ideal candidate for transdermal administration. Therefore, if given through transdermal route, the desired pharmacological effects of rebamipide may be obtained with low doses [compared to high oral dose (80 mg/kg twice a day)].

To serve the purpose of present study, rebamipide-loaded transdermal patches were prepared and evaluated for their physicochemical characteristics and *ex vivo* permeation. Thereafter, the transdermal patches were observed for their potential against 6-OHDA-induced hemiparkinson's rat model as shown in **Figure 9.1**. Motor symptoms of the model were evaluated by a battery of behavioral tests. The

concentration of rebamipide was estimated in plasma and CSF. PD pathology and effects of treatment were evaluated by estimating the levels of DA, TH, α-synuclein concentration, GCase enzymatic activity, DAT levels and Nissl's staining of SNc.

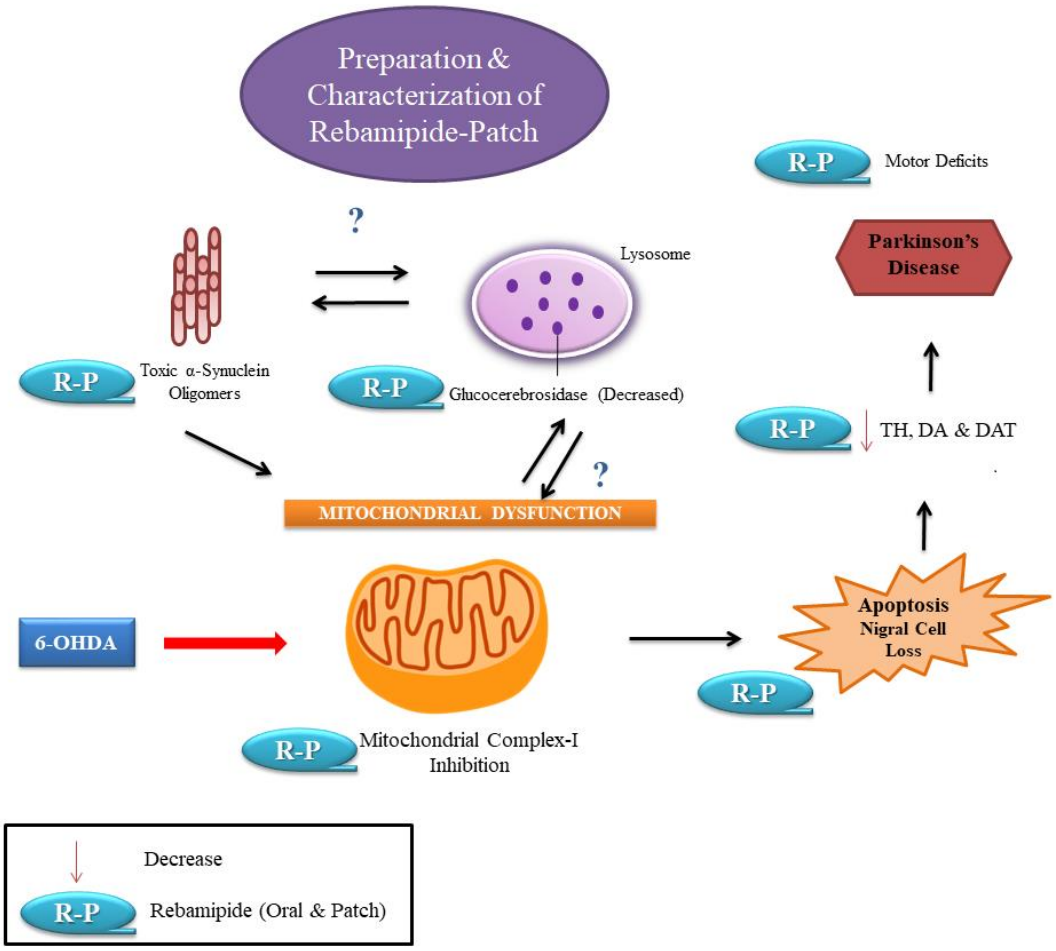

Figure 9.1 The schematic diagram of hypothesis for the design, characterization and evaluation of transdermal patches of rebamipide in rodent model of PD. Rebamipide-containing patches are formulated and characterized for physicochemical characteristics. *Ex vivo* studies are performed, followed by evaluation of patches against 6-OHDA toxicity to compare its potency with oral dose. Rebamipide-containing patches may inhibit mitochondrial dysfunction and α-synuclein pathology, which may be followed by increase in GCase activity, number of Nissl bodies, levels of TH, DA and DAT along with recovery of motor behavior in rats.

9.2. Materials and Methods

9.2.1. Animals

Charles-Foster strain of adult albino rats male (260 ± 20 g) was procured from Central Animal House; Institute of Medical Sciences, Banaras Hindu University (IMS-BHU) and acclimatized at a temperature of 25 ± 1^0C and 45-55% relative humidity with light/dark cycle of 12:12h by keeping them in polypropylene cages. Commercial food pellets (Doodhdhara Pashu Ahar, India) and water was made available *ad libitum.* No experiments were performed for one week in order to let the animals adapt to the laboratory conditions. All the experimental procedures were carried out in compliance with the principles of laboratory animal care [National Institutes of Health guide for the care and use of Laboratory animals (NIH Publication No. 8023, revised 1978)] guidelines and approved by the Institutional animal ethical committee, BHU (Dean/2016/CAEC/33). The experiments were performed between 9:00h and 16:00h.

9.2.2. Materials

HPMC-METHOCEL K100LV-CR premium USP/EP was obtained from Colorcon Asia Pvt. Limited, India. Polyvinyl alcohol (PVA), propylene glycol (PG), polyethylene glycol 400 (PEG400), sodium lauryl sulphate, DMF (N,N-dimethylformamide), tween 80 and isopropyl myristate (IPM) were purchased from Hi-media (Mumbai). Medical adhesive tape USP type was obtained from local suppliers. For the source of remaining materials, refer Chapter 3 (page 18), 4 (page 29), 5 (page 68) and 6 (page 102).

9.2.3. Preparation of backing membrane

Backing membrane was prepared using aqueous solution of PVA (6% w/v). Distilled water (100 mL) was taken in beaker and allowed to heat at 60^0C. PVA (6 gm) was added to the beaker and constantly stirred on a magnetic stirrer at 100 rpm. Stirring was continued for 30 min to attain the homogenous solution without bubble-formation. Homogenous solution (5 mL) was poured into the surface of petridish covered with aluminum foil. The rate of solvent evaporation should be controlled; therefore funnels were kept inverted over the petridish which were placed at 60^0C for 6h to attain transparent, smooth and uniform backing membrane (Malaiya et al., 2018).

9.2.4. Formation of patches over the backing membrane

Transdermal patches are used to deliver a specific dose of drug for extended period of time through the skin, which goes into the bloodstream. Solvent casting method was used to prepare rebamipide-loaded transdermal patches. Rebamipide is classified as BCS Class-IV agent due to its low aqueous solubility and low permeability (Pradhan et al., 2015; Stojančević et al., 2013), therefore permeation enhancers and solubilizing agents were added to prepare the transdermal patches.

Four different formulations were made by altering the ratio of permeation enhancers and polymer. Transdermal patches were prepared using solvent casting method (Nair et al., 2013). HPMC-METHOCEL K100LV was used as polymer due to its suitability for low solubility-drugs (Phadtare, 2014). Firstly, polymer (HPMC-METHOCEL K100LV) was dissolved in solvent by adding in smaller proportions (500 mg at a time) to the beaker gradually and stirred constantly at 500 rpm on a

magnetic stirrer at 50^0C. The composition of patches is shown in **Table 9.1.** Weighed amount of drug [4.0 mg/patch] was dissolved in DMF (penetration enhancer) (Williams and Barry, 2012) and added in the beaker, followed by IPM (permeation enhancer) (Parhi and Padilam, 2018). PEG400 (plasticizer, penetration enhancer) (Chessa et al., 2011; Singh and Bali, 2016) and sodium lauryl sulphate (penetration enhancer) (Som et al., 2012) were added, followed by PG (plasticizer and penetration enhancer) (Chessa et al., 2011; Prabhakara et al., 2010). Tween 80 (surfactant) (Gannu et al., 2010) and transcutol (solubilizing agent, penetration enhancer) (Censi et al., 2012; Chessa et al., 2011) were added to the mixture. The contents were continuously stirred with magnetic stirrer for 20 min in order to get homogenous mixture. The contents were then poured on the prepared backing membrane placed in petridish, and allowed to dry for 24h at room temperature. The complete evaporation of solvent resulted into the formation of uniform and flat patches of rebamipide (2.5 cm diameter). Aluminum foil was used to wrap the dried patches which were stored in silica gel-containing desiccator for further characterization.

Table 9.1 Formulation of Transdermal Patches

S.No.	Formulation Code	Solvent [Ethanol:Water] [1:2]	DMF:IPM [1:9]	Tween 80	Sodium Lauryl Sulphate	Transcutol	HPMC-METHOCEL K100LV	PE 400	PG
1	A	As required [~25 mL]	10% [0.1:0.9 mL]	5% [0.5 mL]	-	10% [1 mL]	55% [5.5 gm]	10% [1 mL]	10% [1 mL]
2	B	As required [~25 mL]	10% [0.1:0.9 mL]	5% [0.5 mL]	-	10% [1 mL]	25% [2.5 gm]	40% [4 mL]	10% [1 mL]
3	C	As required [~25 mL]	10% [0.1:0.9 mL]	2.5% [0.25 mL]	5% [0.5 gm]	7.5% [0.75 mL]	25% [2.5 gm]	40% [4 mL]	10% [1 mL]
4	D	As required [~25 mL]	10% [0.1:0.9 mL]	2.5% [0.25 mL]	5% [0.5 gm]	7.5% [0.75 mL]	55% [5.5 gm]	10% [1 mL]	10% [1 mL]

9.2.5. Physicochemical evaluation of transdermal patches

9.2.5.1. Preformulation studies

- **Phosphate buffer pH 7.4**

Disodium hydrogen phosphate dehydrate (35.61 g) was dissolved in distilled water and the volume was made up to 1000 mL (solution A). Sodium dihydrogen phosphate dihydrate (31.21 gm) was weighed, dissolved in distilled water and volume was made up to 1000 mL (solution B). 405 mL of solution A and 95 mL of solution B were added together and final volume was made 1000 mL with distilled water (Sambrook and Russell, 2001).

- **Calibration curve of rebamipide**

Rebamipide content was analyzed using UV spectrophotometric method. A 100µg/mL standard stock solution of rebamipide was prepared by dissolving 1 mg of drug in 10 mL of 40% v/v PEG400- phosphate buffer (pH 7.4) mixture. Scanning of the stock solution was performed at the UV range (190 to 400 nm) to obtain λmax. Serial dilutions ranging from 2µg/mL to 20µg/mL of rebamipide were prepared by diluting stock solutions. The absorbance values of each dilution were taken at λmax (**230 nm**) against 40% v/v PEG400- phosphate buffer (pH 7.4) mixture taken as the blank, and calibration curve was plotted.

9.2.5.2. Uniformity of weight

Patches were weighed separately on digital weighing machine (three patches per formulation). Average weight was calculated and results were shown as mg (mean ± SD).

9.2.5.3. Patch Thickness

The thickness of the drug-containing patches was measured by using micrometer/digital caliper. Three patches were taken randomly from each formulation to calculate the thickness at three different points of the patch. Average thickness was denoted in mm as mean ± SD.

9.2.5.4. Folding endurance

Patches were evaluated manually for their brittleness and the ability to withstand folding. Patch was folded repeatedly at the same place till it developed visible cracks or got broken. The results were expressed as the number of times patch could be folded at one place without breaking/ cracking (Kusum Devi et al., 2003; Raghuraman et al., 2002). The test denotes the strength of the patch and was performed using three patches of each formulation. Results were denoted as mean ± SD.

9.2.5.5. Surface pH

Double distilled water (0.5 mL) was added to the surface of the patch. It was kept in glass tubes for 1h at room temperature and allowed to swell. The surface of the patch was kept in contact with the pH meter in order to allow the equilibration for 1 min.

Three patches were taken from each formulation to take the average, which was denoted as mean ± SD (Bottenberg et al., 1991).

9.2.5.6. Drug content uniformity

Transdermal patches were evaluated for content uniformity. Drug containing patches were cut into smaller pieces (1 cm^2) and immersed in 10 mL of solvent mixture [40% v/v PEG400- phosphate buffer (pH 7.4)] in beaker to extract the drug in patches. It was then stirred overnight using magnetic stirrer for complete dissolution of polymeric matrices. Centrifugation was performed at 5000 rpm for 30 min. Solution was then filtered with whatman filter paper (0.45 µ), diluted and the absorbance was taken using UV Spectrophotometer (λmax 230 nm). Samples were studied in triplicate, and respective placebo patches (without drug) were taken as blank solution. Drug content of each patch was measured from the absorbance values using standard curve of rebamipide (Parhi and Padilam, 2018; Ramadan et al., 2018). The test was performed in triplicate for each formulation and the mean values were calculated. The results were expressed as % (mean ± SD).

9.2.5.7. Swelling studies

The swelling behavior of drug-containing patches was studied using the method reported previously (Ammar et al., 2009). Transdermal patches were weighed (Wo) by placing on a preweighed petridish and immersed in 25 mL of phosphate buffer (pH 7.4) in beaker. Beakers were placed at 25^0C using thermostated water bath. After every 5 min, the patches were weighed (Wt) after removing the excess surface water by blotting them lightly with a filter paper. The experiment was discontinued once the patches began to dissolve or erode. The results were expressed as % (mean ± SD).

The swelling index of the patch due to the presence of polymer was calculated as follows:

$$\%Swelling = \frac{Wt - Wo}{Wo} \times 100$$

9.2.5.8. Percentage moisture loss

Moisture loss was performed to observe the integrity of transdermal patches under dry conditions. Three patches ($1cm^2$) of each formulation were weighed and kept in a silica gel-containing desiccator at room temperature. During this period, the transdermal patches were weighed at specific time intervals of 24, 48 and 72h until they showed constant weight (Ubaidulla et al., 2007). The results were expressed as % (mean ± SD). Percentage moisture loss was calculated using the formula:

$$\%MoistureLoss = \frac{InitialWeight - FinalWeight}{FinalWeight} \times 100$$

9.2.6. *Ex vivo* drug permeation studies

Drug permeation studies were carried out using the skin of rats. Skin was excised from the dorsal region of rat after killing them by cervical dislocation. The skin samples were washed using normal saline. Adhering fat and connective tissue were removed using blunt-end forceps. The skin was placed in normal saline solution at 4^0C for 6h. The skin was shaved carefully and hairs were removed. Drug permeation studies were performed using Franz diffusion cells. Drug-containing transdermal patches were kept adhered to the stratum corneum of the skin in the donor compartment of diffusion cell. 40% v/v PEG400 - phosphate buffer (pH 7.4) mixture 20 mL was filled in the receptor compartment of the diffusion cell. The temperature

of the assembly was constantly maintained at 37 ± 0.5^0C and stirred with small magnetic spin bar (50 rpm). Samples (1 mL aliquots) were withdrawn at predetermined time points (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 24h) from the receptor compartment, and replaced with an equal volume of fresh medium [40% v/v PEG400 - phosphate buffer (pH 7.4) mixture] to maintain the receptor phase volume to 20 mL. The samples collected from the receptor compartment were analyzed by using UV spectrophotometer (λmax 230 nm) to measure the concentration of rebamipide (Nair et al., 2013).

9.2.7. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was employed to analyze the pure drug rebamipide as well as drug-containing transdermal patches. This was performed to investigate any incompatibility between drug and polymer of patches in the frequency range of 4000-600 cm^{-1} at room temperature. The spectra of prepared samples were recorded by directly placing the samples in the instrument (Parhi and Suresh, 2016).

9.2.8. Surface Morphology

Surface morphology of patches was visualized using scanning electron microscope (SEM, EVO LS 10, Carl Zeiss, Germany) at an acceleration voltage of 10 kV.

9.2.9. Skin irritation studies

The study was performed by using healthy adult albino male rats of Charles-Foster strain (weighing 250-300 g, n = 6). Their dorsal abdominal skin was shaved carefully avoiding any peripheral damage at 24h before conducting the study. The hairless skin was cleaned with warm water and the transdermal patch was applied using medical

adhesive tape USP type. Rats were randomly divided into 4 groups, namely, control (which were left untreated), adhesive tape USP type (rats were applied with adhesive tape), formalin (rats were applied with standard skin irritant formalin, aqueous solution 0.8% v/v), rebamipide transdermal patch (R-patch, rats were applied with drug-loaded patches). Patches were removed after 48h using alcohol swab. Skin irritation was evaluated on the basis of scoring method (Draize et al., 1944). Rats were scored from 0 to 4 based on the severity of erythema. Erythema scale: score 0, none; score 1, very slight (light pink); score 2, well-defined (dark pink); score 3, moderate (light red); score 4, severe and scar formation (dark red) (Sarkar et al., 2014).

9.2.10. *In vivo* studies

9.2.10.1. Surgery and Microinjection

Please refer Chapter 4 (page 30).

9.2.10.2. Experimental Design

Rats were randomly divided into six groups (twelve rats in each group), namely control, sham, 6-OHDA, 6-OHDA+Selegiline, 6-OHDA+ R-Oral (rebamipide at 80 mg/kg twice daily oral), 6-OHDA+R-Patch (one transdermal patch of rebamipide daily). 6-OHDA intrastriatal injection was given to rats on D-1 into the left striatum except control and sham groups. For control and sham groups, please refer Chapter 6 (page 103). Selegiline was given as 10 mg/kg orally and used as positive control in present study (Mishra et al., 2018). Rebamipide suspension (80 mg/kg) was administered orally twice daily at every 12h, as discussed in Chapter 6 (page 103)

(Mishra and Krishnamurthy, 2019). Drug administration was initiated to their respective groups after the onset of motor deficits from D-4 and continued up to D-27 after 6-OHDA intrastriatal injection. To confirm 6-OHDA-induced motor deficits, apomorphine-induced head rotation test was conducted on D-4. The hair of dorsal abdominal skin was shaved carefully avoiding any peripheral damage at 24h before applying the transdermal patches. On D-4, the hairless skin was cleaned with warm water and the rebamipide-loaded transdermal patch was applied. Medical adhesive tape USP type was used to fix the patch on dorsal nude skin (Ramadan et al., 2018). The oral bioavailability of rebamipide is reported to be 4.8 ± 1.4% (Shin et al., 2004). Therefore, daily absorbed oral dose of rebamipide will be ~8 mg/kg, when given 80 mg/kg orally twice daily. Since, epidermis is metabolically inert (bioavailable factor = 1) (Chandrashekar and Rani, 2008), the transdermal patches were prepared as 8 mg/kg dose. For rats weighing 300 g, 80 mg/kg oral dose of rebamipide twice a day will be equivalent to 2.4 mg rebamipide-containing transdermal patches once a day. Since a drug should be present in a relatively high dose inside the patch, which is applied on the skin for the longer period of time (Sheth and Mistry, 2011). Therefore, patches were prepared as 4.0 mg rebamipide/patch, each having 2.5 cm diameter. Rats were applied with new patch daily on dorsal abdominal nude skin up to D-27 after 6-OHDA intrastriatal injection, by removing the previous patches with the help of alcohol swab. All the behavioral parameters were performed on D-0, 7, 14, 21, 28. Training sessions for behavioral parameters were performed as discussed in Chapter 4 (page 32-33). Behavioral tests were recorded with a video camera by observers blind to the study protocol. After 12 hours of last oral dose administration of rebamipide, blood samples (0.25 mL) were taken in pre-labeled heparin-coated

sampling tubes at D-28 through retro-orbital plexus (6-OHDA+ R-Oral and 6-OHDA+ R-Patch groups; n = 6). Rats were given equal volume of normal saline through i.p. route after each blood withdrawal. Blood-containing tubes were centrifuged at 4000 rpm for 10 min at 4^0C. Plasma was collected and stored at -80^0C until analysis (Muddana et al., 2014). On D-28, CSF was also collected. Plasma and CSF samples were extracted by taking sample aliquots (100 µL) in micro centrifuge tubes and mixed with similar amount of methanol. Mixture was shaken in the vortex mixer for 30s and centrifuged at 12000 rpm for 10 min at 4^0C. The supernatant was collected and transferred into chromatographic vials for further HPLC analysis. Apart from the six animals per group assigned for Nissl's staining (n = 6), remaining of the animals were killed by cervical dislocation on D-28. Nigral and striatal tissues from ipsilateral hemispheres were micro dissected (Paxinos and Watson, 1998) and stored at -80^0C. TH levels, α-synuclein concentration and GCase activity were measured in nigral tissues (n = 6). Striatal tissues were used to measure DA and DAT levels (n = 6). Detailed experimental design for *in vivo* studies is depicted in **Figure 9.2**.

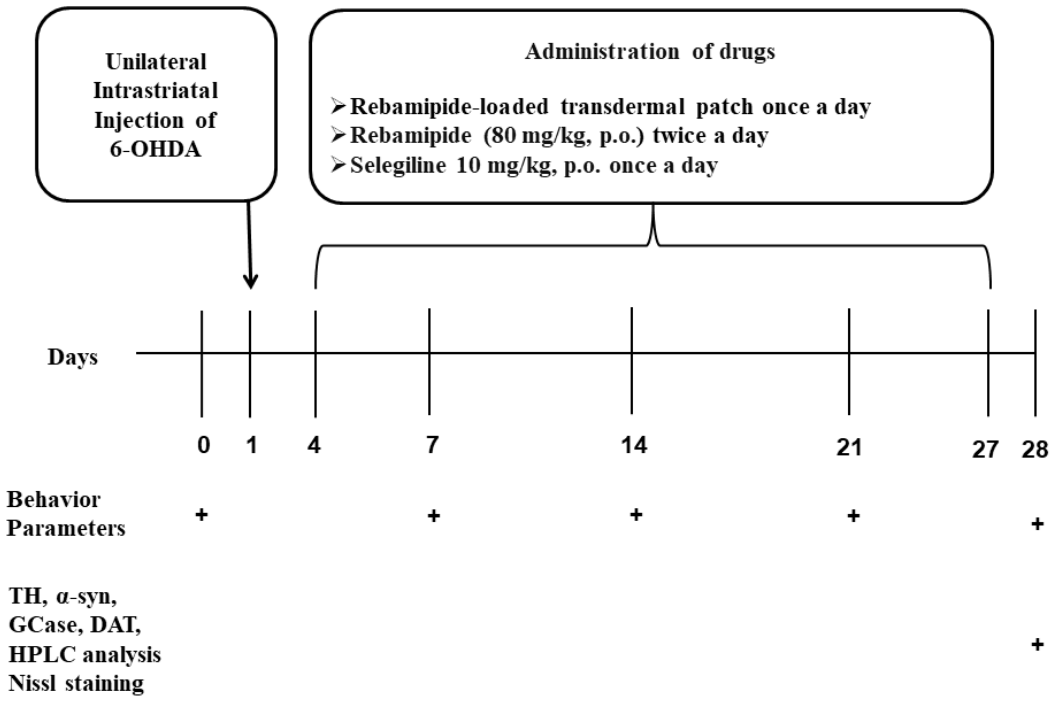

Figure 9.2 Experimental design for the evaluation of transdermal patches of rebamipide in rodent model of PD. "+" indicates days at which the tests were performed.

9.2.10.3. Behavioral parameters

Please refer Chapter 4 (page 34-35) for rotarod retention time, apomorphine-induced head rotation, bar catalepsy and grip strength tests.

9.2.10.4. Measurement of DA, TH, DAT and soluble α-synuclein levels

Please refer Chapter 4 (page 37) and Chapter 6 (page 106) to measure the levels of TH and α-synuclein in ipsilateral nigral tissues and DAT along with DA in ipsilateral striatal tissues.

9.2.10.5. Measurement of GCase activity

GCase activity was estimated in ipsilateral nigral tissues as described in Chapter 4 (page 36).

9.2.10.6. Quantification of rebamipide (HPLC analysis)

The analyses were carried out using high-performance liquid chromatography (HPLC-Agilent 1260 infinity II Quaternary LC). Quaternary pump with flow rate set at 1 mL/min was used to deliver isocratic mobile phase, which included water as phase A, acetonitrile as phase B and methanol as phase C in ratio of 5:3:2. Samples (5µL) were injected into the HPLC column through auto sampler. DAD HS G7115A (Diode-array detector) was used at 230 nm for detecting rebamipide in plasma and CSF. Agilent ZORBAX Eclipse plus C8 column (5 µm, 4.6 × 250 mm) was used (Manglani et al., 2006; Shi et al., 2013). The concentration of drug was calculated using standard curve (Cooper and Harirforoosh, 2014).

9.2.10.7. Nissl's staining

Please refer Chapter 4 (page 38).

9.2.11. Statistical Analysis

The results were expressed as mean ± SD. *Ex vivo* permeability and behavior parameters were analyzed by repeated measures of two-way ANOVA, followed by Bonferroni post-hoc test. Remaining *in vivo* parameters, physicochemical characteristics (weight, thickness, folding endurance, surface pH, swelling, moisture loss and drug content uniformity) and skin irritation studies was analyzed by one-way ANOVA followed by Student-Newman-Keuls post-hoc test. Rebamipide

concentration using HPLC was analyzed by unpaired t test. $p < 0.05$ was considered significant throughout the analysis.

9.3. Results

9.3.1. Physicochemical characterization of Rebamipide

9.3.1.1. Preformulation studies:

As shown in absorption spectra of rebamipide **(Figure 9.3)**, the λmax was obtained at wavelength of 230 nm **(Table 9.2).** Calibration curve of rebamipide (at λmax 230 nm) is shown in **Figure 9.4.**

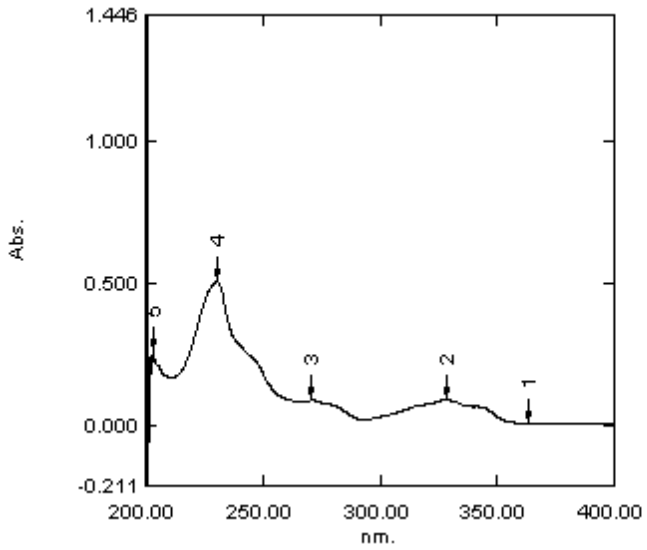

Figure 9.3 Absorption spectra of rebamipide

Table 9.2 Absorbance values of rebamipide solution at different wavelengths, obtained from absorption spectra (Figure 53)

S. No.	Wavelength	Absorbance
1	363.20	0.010
2	328.40	0.091
3	270.60	0.089
4	230.60	0.508
5	203.00	0.264

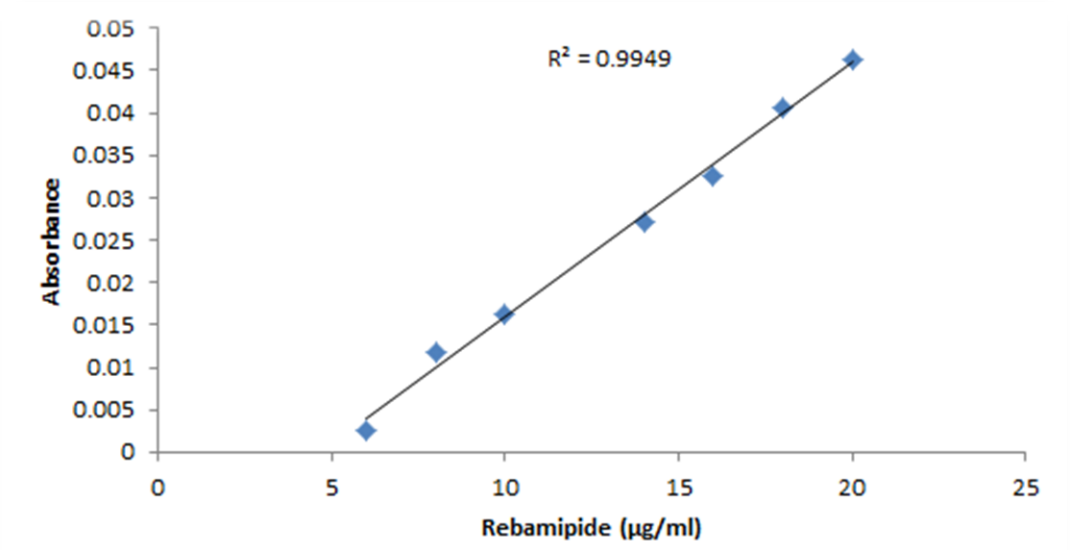

Figure 9.4 Calibration curve of rebamipide

9.3.1.2. Uniformity of thickness, folding endurance, surface pH and drug content

Four different formulations were made by altering the ratio of permeation enhancers and polymer **(Figure 9.5)**. All the patches were observed for their physical parameters and found smooth, flexible and uniform**.** All the four type of formulations were observed to be uniform in their folding endurance, thickness, surface pH and drug content with lower SD values. Thickness of the patches ranges from 00.43 to 00.59 mm (± 0.02 to ± 0.08) **(Table 9.3).** The surface pH was observed as 5.43 to 5.67 (± 0.06 to ± 0.12). The SD values are minimal, indicating the minimum intra batch variability, uniformity and reproducibility. The folding endurance value was observed to be 291-310 (± 6 to ± 11), showing flexible with good tensile strength enough to withstand the mechanical pressure. It also indicates that patches when applied on the skin will be able to maintain the integrity with skin folding (Nair et al., 2013). Nearly uniform drug content was observed for the patches, which ranges from 93.97% to 98.41%. Minimum SD values also assured that the procedure used to formulate the patches have the potential to generate reproducible results with nearly constant drug content showing minimum variability.

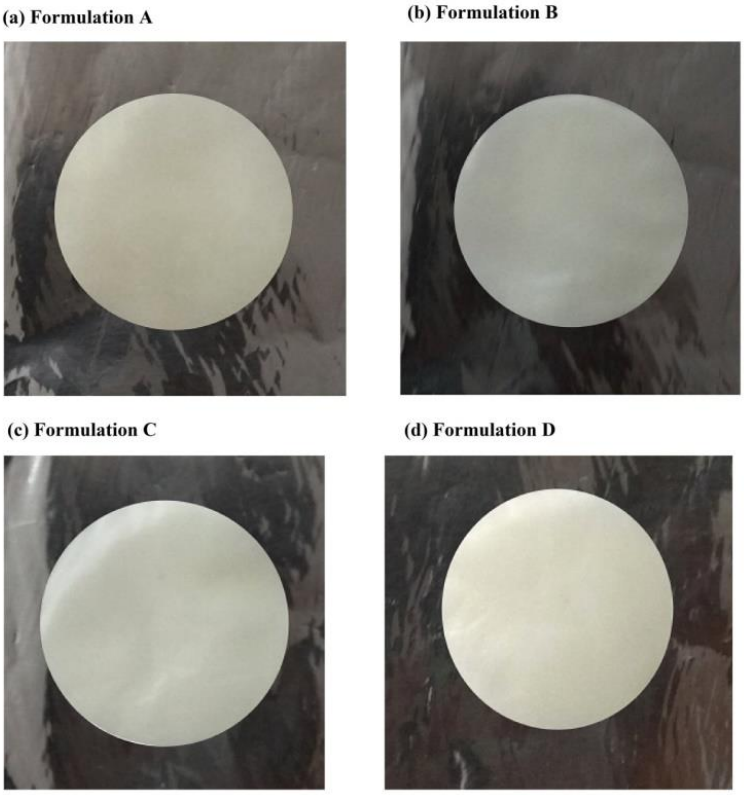

Figure 9.5 Rebamipide-containing Transdermal Patches. Formulation A (a), Formulation B (b), Formulation C (c) and Formulation D (d).

Table 9.3 Physicochemical parameters (thickness, folding endurance, surface pH and drug content) of the formulated patches containing rebamipide

S.No.	Formulation Code	Patch Thickness (mm)	Folding endurance	Surface pH	Drug content uniformity (%)
1	A	00.58 ± 0.02	295 ± 10	5.43 ± 0.12	95.65 ± 3.11
2	B	00.46 ± 0.06	306 ± 8	5.51 ± 0.08	93.97 ± 2.43
3	C	00.51 ± 0.08	291 ± 6	5.49 ± 0.06	98.41 ± 2.92
4	D	00.45 ± 0.03	310 ± 11	5.67 ± 0.10	94.85 ± 3.39

All values are mean ± SD; n = 3.

9.3.1.3. Weight and swelling behavior

Weight of patches varied from 43.12 mg to 59.46 mg (± 0.35 to ± 0.51). All the patches were significantly different from each-other in terms of weight due to different composition of polymers. One-way ANOVA revealed significant differences in weight [$F(3, 8) = 768.2$; $p < 0.05$] and swelling behavior [$F(3, 8) = 17.51$; $p < 0.05$] among formulations as shown in **Table 9.4**. Swelling behavior of transdermal patches is essential to get uniform patches with prolonged drug release and also for appropriate skin adhesion. Empty spaces are formed within the patch due to water-uptake and make the structure of less resistant to mechanical stresses (Malaiya et al., 2018). Swelling behavior of the patches ranges from 61.55 ± 2.76% to 73.28 ± 2.11%. The considerable swelling ability shows the hydrophilic nature of used polymer. The swelling of formulations A and D is significantly high due to presence of increased concentration of polymer compared to formulations B and C. Increased swelling behavior increase the surface wettability, followed by water penetration within the matrix (Malaiya et al., 2018).

9.3.1.4. Moisture loss studies

These studies were conducted for evaluation of the stability of the prepared patches under dry ambient conditions. The % moisture loss of all the formulated patches ranges from 2.01 to 3.42%, which reflects lower moisture loss in the transdermal patches with formulation C showing minimum loss and formulation A showing maximum moisture loss. One-way ANOVA revealed significant differences in % moisture loss [$F(3, 8) = 661.2$; $p < 0.05$] among formulations as shown in **Table 9.4**. All the patches are significantly different to each-other in terms of % moisture loss.

However, the moisture loss was found to be significantly lower in patches formulated with high concentration of PEG400 (formulations B and C) compared to formulations A and D, which indicates that the plasticizer takes part in making the formulation stable with less brittleness under dry conditions during long term storage.

Table 9.4 Physicochemical parameters (weight, swelling and moisture loss) of the formulated patches containing rebamipide

S.No.	Formulation Code	Patch Weight (mg)	Swelling (%)	Moisture loss (%)
1	A	51.52 ± 0.51	70.81 ± 2.48	3.42 ± 0.01
2	B	43.12 ± 0.35[a]	64.53 ± 1.42[a]	2.34 ± 0.04[a]
3	C	48.87 ± 0.39[a,b]	61.55 ± 2.76[a]	2.01 ± 0.03[a,b]
4	D	59.46 ± 0.43[a,b,c]	73.28 ± 2.11[b,c]	3.04 ± 0.07[a,b,c]

All values are mean ± SD; n = 3; [a]$p < 0.05$ compared to formulation code A, [b]$p < 0.05$ compared to formulation code B, and [c]$p < 0.05$ compared to formulation code C [one-way ANOVA followed by post hoc Student Newman-Keuls test].

9.3.2. *Ex vivo* drug permeation studies

Ex vivo permeation and release profile is a significant tool to predict the behavior of drug *in vivo* in terms of duration and rate of drug action. The drug permeation in 24h as cumulative percentage was observed to be satisfactory for all the formulation and drug permeation ranged from 66.06% (formulation A) to 91.46% (formulation C). Repeated measures of two-way ANOVA showed significant differences in

cumulative drug permeated among different formulations [$F(3, 120) = 80.09$; $p < 0.05$], time [$F(14, 120) = 260.8$; $p < 0.05$] and an interaction [$F(42, 120) = 1.590$; $p < 0.05$] between formulations and time **(Figure 9.6)**. Rebamipide was observed to permeate through the skin membrane in 24h. The release profile of formulation A with formulations B and D were not found to be significantly different at any time point. Nearly 50% drug of formulations C and D was released up to 8h which is equivalent to half-life of rebamipide (7.48 ± 0.92 h) in rats (Cooper et al., 2014). However, the overall drug permeation release of formulation C at 24h was found to be significantly highest (91.46%) compared to all other formulations (A 66.06%, B 69.61%, and D 75.68%). The improved drug release profile of formulation C may be due to the lower concentration of polymer, high concentration of plasticizer (PEG400) and addition of multiple permeation enhancers compared to other formulations. The enhanced matrix system due to high concentration of polymer (HPMC-METHOCEL K100LV) results into enhanced resistance to the drug release from the patches. PEG400 being hydrophilic plasticizer not only increases mechanical strength of the patches but also weakens the matrix structure against aqueous medium (Ramadan et al., 2018). Therefore, the polymer was hydrated easily, swelled and results into faster and extended release of drug.

9.3.3. FTIR spectroscopy

The pure drug in its FTIR spectrum showed characteristic sharp peaks at 1725.46cm^{-1} due to aldehydes (C=O) stretch and at 1642.98 cm^{-1} due to C=O-NH stretching. Stretching vibrations were also observed due to primary and secondary amine at 1594.06 cm^{-1} and 1542.31-1505.13 cm^{-1}. Vibrations for –C-H rock alkanes and –C-H

bend alkanes stretching were observed at 754.11 cm^{-1} and 1428.70 cm^{-1} respectively **[Figure 9.7 (a)].** No novel significant peaks were observed in the drug-loaded transdermal patches **[Figure 9.7 (b)],** confirming the absence of any interaction between the drug polymers. The characteristic peaks of rebamipide appeared with no significant shifting in the FTIR spectra of rebamipide-loaded transdermal patch, which indicates that the identity of rebamipide was maintained after the preparation of HPMC-METHOCEL K100LV - based transdermal patch.

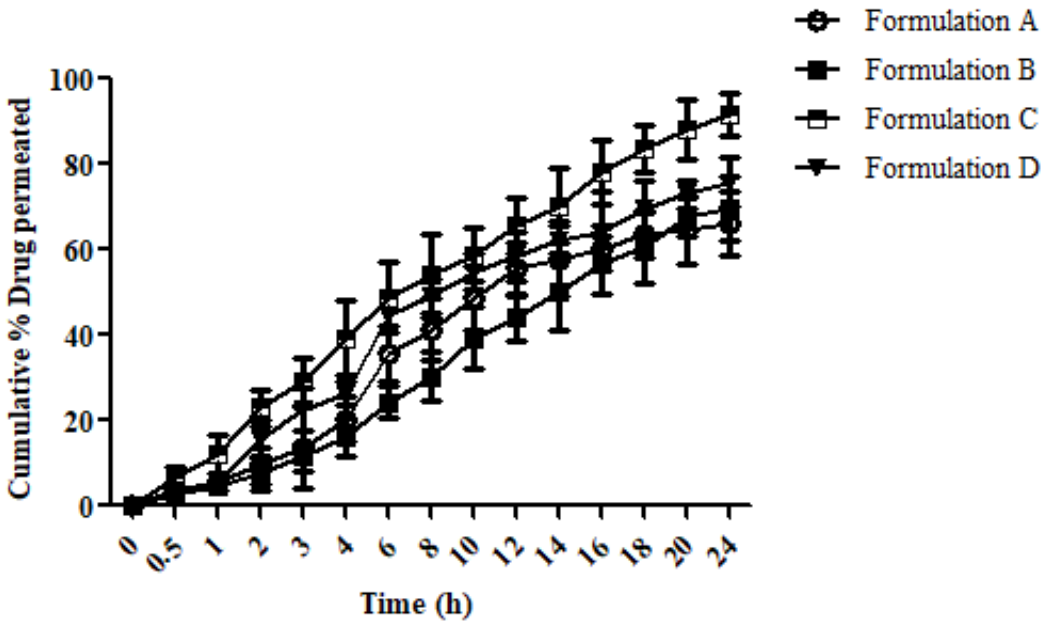

Figure 9.6 ***Ex vivo*** **drug permeation profiles of formulations (rebamipide-loaded transdermal patches) A, B, C and D through rat skin** (mean ± SD; n = 3).

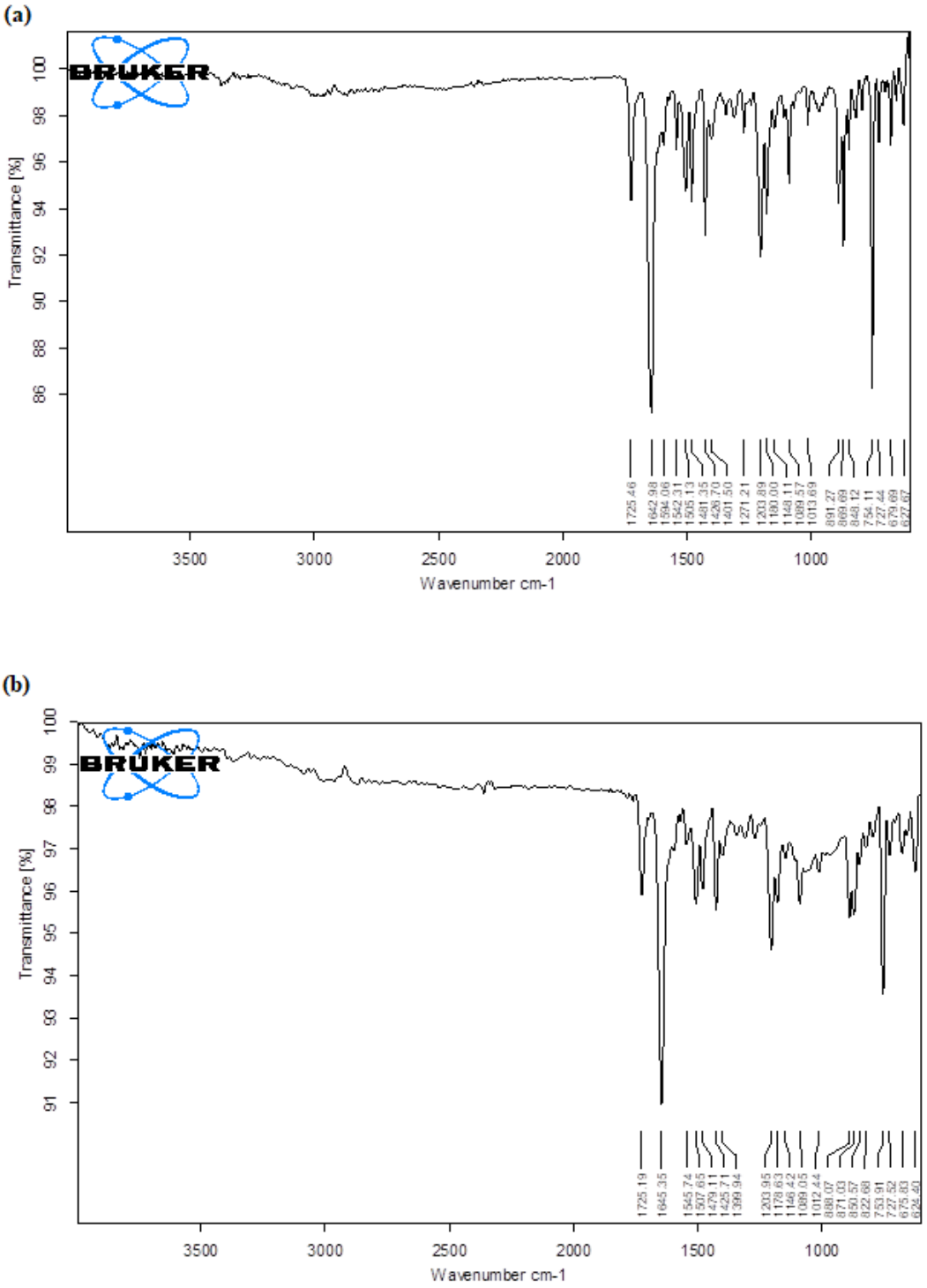

Figure 9.7 FTIR Spectra of pure drug rebamipide (a), and rebamipide-loaded transdermal patch formulation C (b).

9.3.4. Surface Morphology

Photomicrographs depict the SEM images of pure drug and patches at different magnifications **(Figure 9.8).** Rebamipide crystals were observed. The surface morphology of patch having best *ex vivo* permeability (formulation C) at different magnifications confirmed the compatibility of rebamipide with transdermal patch. This showed the appearance of homogenous film with the smooth surface.

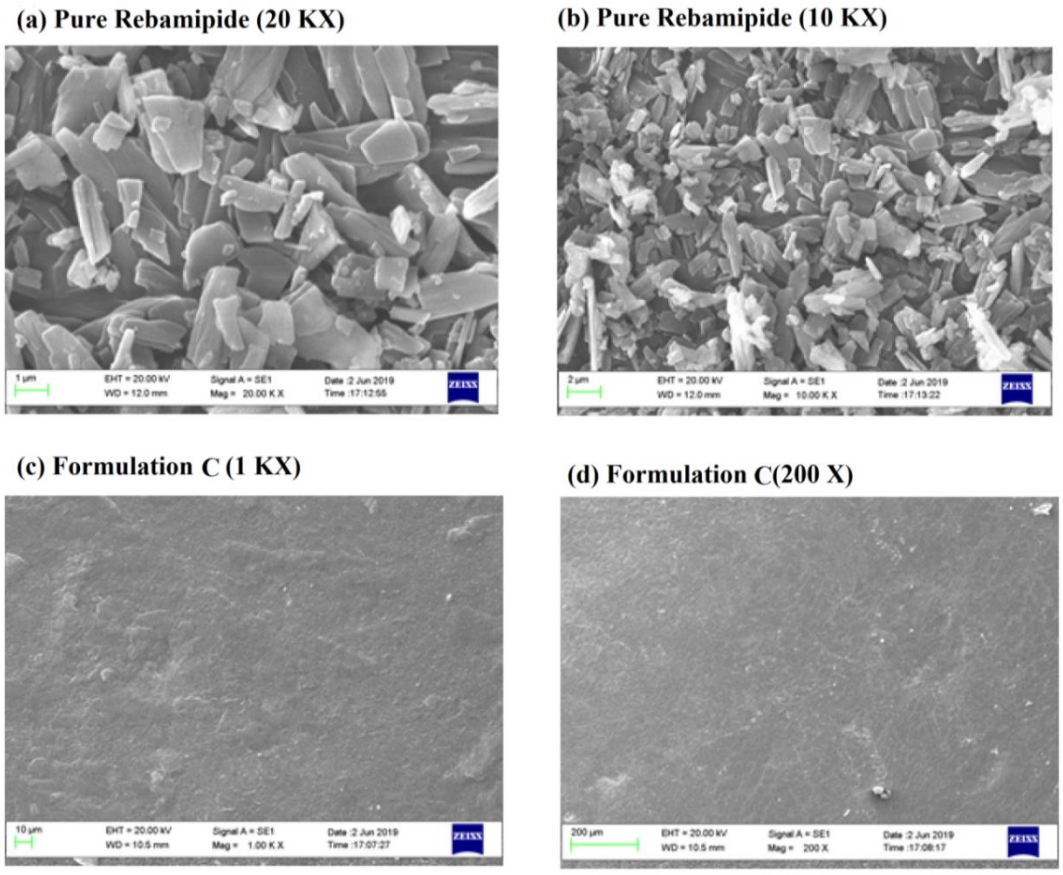

Figure 9.8 SEM images of pure rebamipide (a, b), and rebamipide-loaded transdermal patch formulation C (c, d) at different magnifications.

9.3.5. Skin irritation studies

Biocompatibility of transdermal patches with the site of action (skin) is of utmost importance as they are applied topically (Arunachalam et al., 2010). There should be no skin irritation, sensitization, hypersensitivity or inflammatory reactions, which may lead to the redness or flushing of skin. Skin irritation was evaluated on the basis of scoring method (Draize et al., 1944). Rats were scored from 0 to 4 based on the severity of oedema or erythema (Sarkar et al., 2014). Formulation C was tested on rats and none of the group was shown to demonstrate severe erythema, except for the standard formalin group. One-way ANOVA revealed significant differences in erythema scores [$F(3, 20) = 401.8$; $p < 0.05$] among groups as shown in **Table 9.5**. The erythema scores due to rebamipide patches were found to significantly different than control, adhesive tape USP type and formalin groups. The erythema scores due to adhesive tape USP type and rebamipide patches were observed as 0.29 ± 0.06 and 0.94 ± 0.11 respectively, which indicates very slight erythema. This suggests non-irritant and non-allergenic profile of the developed transdermal patches. Absence of any noticeable irritation on the rat skin indicates skin compatibility of drug and polymer matrix.

Table 9.5 Visual observation for skin irritation test on rat skin

S.No.	Groups	Erythema Score
1	Control	0.00 ± 0.00
2	Adhesive tape USP type	0.29 ± 0.06
3	R-patch	$0.94 \pm 0.11^{a,b}$
4	Formalin (0.8% v/v)	$3.81 \pm 0.41^{a,b,c}$

All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to Control, $^{b}p < 0.05$ compared to Adhesive tape USP type, and $^{c}p < 0.05$ compared to R-patch[one-way ANOVA followed by post hoc Student Newman-Keuls test].

9.3.6. *In vivo* Studies

9.3.6.1. Rebamipide containing transdermal patches attenuated 6-OHDA-induced motor deficits in rats

Repeated measures of two-way ANOVA revealed significant differences in rotational and cataleptic behavior as well as rotarod retention time and grip strength scores in rats among groups ([F (5, 330) = 133.5; $p < 0.05$], [F (5, 330) = 139.8; $p < 0.05$], [F (5, 330) = 164.8; $p < 0.05$] and [F (5, 330) = 121.6; $p < 0.05$] respectively), time ([F (4, 330) = 60.17; $p < 0.05$], [F (4, 330) = 114.8; $p < 0.05$], [F (4, 330) = 151.2; $p < 0.05$] and [F (4, 330) = 120.7; $p < 0.05$] respectively) and an interaction ([F (20, 330) = 24.78; $p < 0.05$], [F (20, 330) = 34.21; $p < 0.05$],[F (20, 330) = 20.41; $p < 0.05$] and [F (20, 330) = 19.24; $p < 0.05$] respectively) between group and time **(Table 9.6).** 6-OHDA increased apomorphine-induced rotational behavior from D-7 (38%) and cataleptic behavior from D-14 (60%) compared to sham group. 6-OHDA decreased grip strength scores (72%) and rotarod retention time (48%) from D-7. The motor

deficits caused by 6-OHDA were found to be progressive. No significant differences were observed between control and sham groups. Both the rebamipide-oral and rebamipide-patch groups significantly attenuated 6-OHDA-induced motor deficits from D-21 in cataleptic behavior (38% and 31% respectively), rotarod retention time (31% and 26% respectively), and grip strength scores (56% and 53% respectively) and from D-14 in rotational behavior (22% and 16% respectively). Rebamipide-patch group was not found to be significantly different than rebamipide-oral group, indicating their similar efficacy and high potency of patches against 6-OHDA toxicity for motor behavior.

9.3.6.2. Rebamipide-containing transdermal patches inhibited 6-OHDA-induced reduction in nigral TH and striatal DA levels in rats

One way ANOVA revealed significant differences in nigral TH [$F(5, 30) = 29.46$; $p < 0.05$] and striatal DA levels [$F(5, 30) = 52.62$; $p < 0.05$] among groups, as depicted in **Figure 9.9 (a) and (b)**. No significant difference was observed between control and sham groups. TH and DA levels were significantly reduced (58% and 66% respectively) by 6-OHDA compared to sham group. Rebamipide both in oral and transdermal administration increased the TH levels (50% and 54% respectively) and DA levels (53% and 48% respectively) against 6-OHDA-infused groups without any significant difference between them. Both the patches and oral dose showed same pharmacological efficacy. However, patches were found to be more potent.

Table 9.6 Effects of rebamipide (oral and transdermal) on 6-OHDA-induced alterations in motor functions as assessed by apomorphine-induced rotations, cataleptic behavior, grip strength score and rotarod retention time in rats

Groups	Apomorphine-induced rotations (Counts/5 min)	Cataleptic Behavior (sec)	Grip Strength Score	Retention Time in Rotarod Test (sec)
DAY 0				
Control	5.17 ± 0.95	1.69 ± 0.46	4.05 ± 0.88	180.12 ± 14.03
Sham	5.30 ± 1.26	1.75 ± 0.44	3.97 ± 0.82	181.12 ± 13.82
6-OHDA	5.06 ± 0.96	1.52 ± 0.39	4.09 ± 0.65	177.87 ± 18.87
6-OHDA+Selegiline	5.34 ± 1.25	1.71 ± 0.40	4.03 ± 0.75	179.09 ± 15.93
6-OHDA+R-Oral	5.31 ± 0.56	1.83 ± 0.49	3.95 ± 0.71	178.70 ± 15.49
6-OHDA+R-Patch	5.40 ± 0.98	1.90 ± 0.45	3.90 ± 0.76	175.89 ± 15.93
DAY 7				
Control	5.61 ± 1.01	1.59 ± 0.42	3.96 ± 0.67	180.52 ± 10.30
Sham	5.07 ± 0.45	1.76 ± 0.36	3.81 ± 0.71	168.03 ± 8.26
6-OHDA	8.20 ± 1.47[a]	1.81 ± 0.20	1.06 ± 0.20[a]	86.37 ± 25.47[a]
6-OHDA+Selegiline	8.21 ± 0.89[a]	1.70 ± 0.23	1.22 ± 0.23[a]	88.06 ± 28.17[a]
6-OHDA+R-Oral	7.81 ± 1.58[a]	1.91 ± 0.51	1.19 ± 0.15[a]	79.65 ± 22.12[a]
6-OHDA+R-Patch	7.89 ± 1.22[a]	1.77 ± 0.44	1.28 ± 0.19[a]	85.88 ± 22.79[a]
DAY 14				
Control	5.24 ± 1.08	1.49 ± 0.39	3.83 ± 0.68	181.62 ± 11.30
Sham	5.38 ± 0.87	1.54 ± 0.40	3.74 ± 0.89	169.53 ± 10.00
6-OHDA	10.84 ± 0.92[a]	3.86 ± 0.89[a]	0.99 ± 0.27[a]	78.32 ± 18.98[a]
6-OHDA+Selegiline	9.35 ± 0.75[a,b]	2.79 ± 0.72[a,b]	2.64 ± 0.35[a,b]	119.17 ± 13.69[a,b]
6-OHDA+R-Oral	8.40 ± 1.51[a,b]	3.63 ± 0.68[a,c]	1.15 ± 0.18[a]	88.32 ± 11.92[a,c]
6-OHDA+R-Patch	9.06 ± 1.03[a,b]	3.92 ± 0.71[a,c]	1.05 ± 0.14[a]	92.96 ± 17.38[a,c]
DAY 21				
Control	5.29 ± 0.75	1.72 ± 0.44	3.89 ± 0.84	180.02 ± 14.02
Sham	5.43 ± 0.69	1.77 ± 0.35	3.77 ± 0.96	171.13 ± 13.64
6-OHDA	12.72 ± 0.98[a]	5.64 ± 0.92[a]	1.20 ± 0.24[a]	92.07 ± 24.09[a]
6-OHDA+Selegiline	5.86 ± 0.58[b]	1.87 ± 0.34[b]	3.85 ± 0.72[b]	165.33 ± 12.34[b]
6-OHDA+R-Oral	7.16 ± 1.64[a,b,c]	3.51 ± 0.55[a,b,c]	2.73 ± 0.52[a,b,c]	132.89 ± 21.67[a,b,c]
6-OHDA+R-Patch	8.27 ± 1.10[a,b,c]	3.86 ± 0.62[a,b,c]	2.54 ± 0.30[a,b,c]	124.18 ± 24.57[a,b,c]
DAY 28				
Control	5.61 ± 0.83	1.81 ± 0.34	3.83 ± 0.94	179.82± 15.31
Sham	5.67 ± 1.04	1.76 ± 0.34	3.84 ± 0.71	171.13 ± 14.11
6-OHDA	13.30± 2.05[a]	5.29 ± 0.51[a]	1.09 ± 0.26[a]	85.31 ± 22.70[a]
6-OHDA+Selegiline	5.92 ± 0.48[b]	2.04 ± 0.32[b]	3.87 ± 0.47[b]	163.86 ± 13.58[b]
6-OHDA+R-Oral	5.90 ± 1.95[b]	2.09 ± 0.17[b]	3.74 ± 0.79[b,c]	161.70 ± 12.15[b]
6-OHDA+R-Patch	4.86 ± 1.23[b]	2.40 ± 0.32[a,b]	3.44 ± 0.29[b,c]	150.85 ± 23.28[a,b]

All values are mean ± SD; n = 12; [a]$p < 0.05$ compared to sham, [b]$p < 0.05$ compared to 6-OHDA, and [c]$p < 0.05$ compared to 6-OHDA+Selegiline [Repeated measures of two-way ANOVA followed by Bonferroni test].

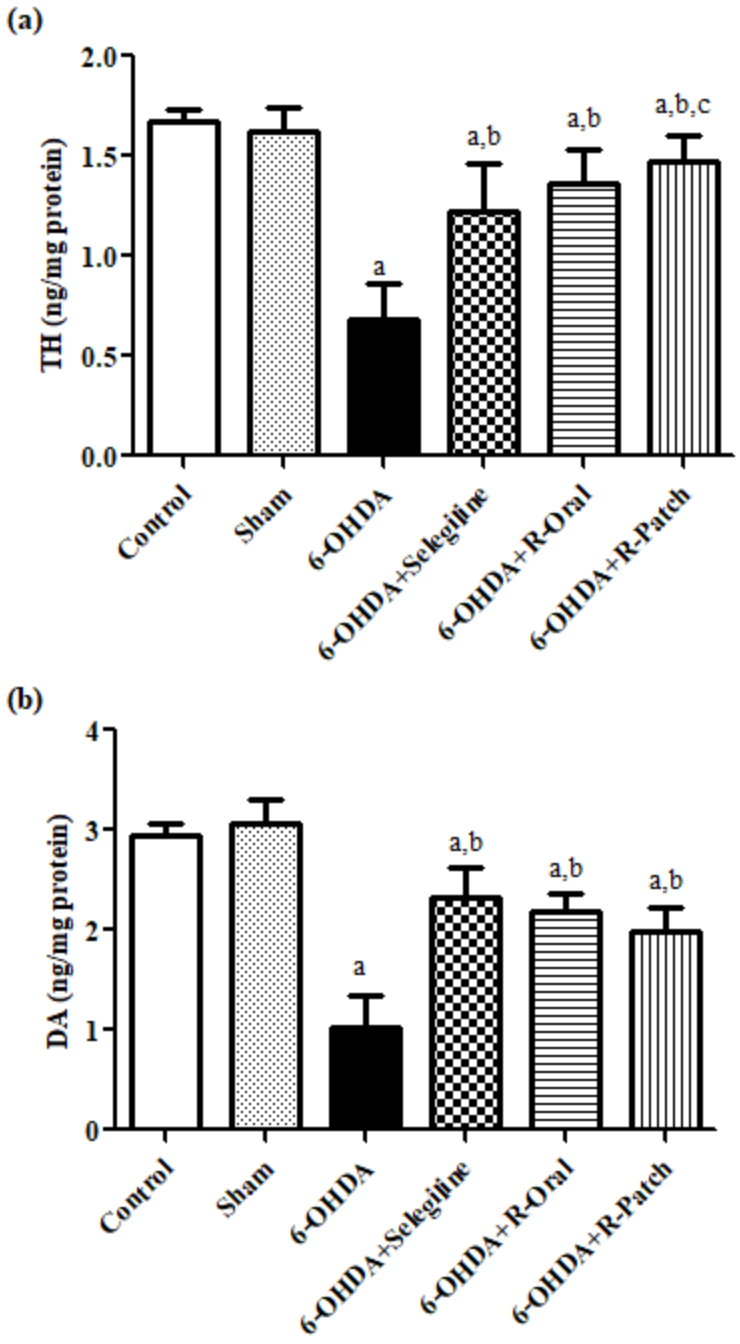

Figure 9.9 Effect of rebamipide (oral and transdermal patches) on 6-OHDA-mediated loss of TH and DA levels in ipsilateral nigral and striatal tissues of rats respectively. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, $^{b}p < 0.05$ compared to 6-OHDA, and $^{c}p < 0.05$ compared to 6-OHDA+Selegiline [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

9.3.6.3. Transdermal patches of rebamipide inhibited 6-OHDA-induced alterations in GCase activity and soluble α-synuclein concentration in nigral tissues of rats

One way ANOVA revealed significant differences in GCase enzymatic activity [$F(5, 30) = 19.60$; $p < 0.05$] and soluble concentration of α-synuclein [$F(5, 30) = 27.19$; $p < 0.05$] among groups. Control and sham groups were not reported to be significantly different as shown in **Figure 9.10 (a)** and **(b)**. 6-OHDA significantly decreased nigral GCase activity (72%) and soluble concentration of α-synuclein (67%) compared to sham groups. Rebamipide both in oral and transdermal patches increased GCase activity (68% and 62% respectively) and soluble α-synuclein concentration (51% and 58% respectively) up to similar extent compared to 6-OHDA group, suggesting their similar pharmacological efficacy and high potency of patches.

9.3.6.4. Rebamipide-containing transdermal patches inhibited 6-OHDA-induced reduction in striatal DAT levels in rats

One-way ANOVA showed significant differences among groups in striatal DAT levels [$F(5, 30) = 20.19$; $p < 0.05$]. 6-OHDA decreased the striatal DAT levels (50%) compared to sham group, indicating decreased viability of dopaminergic cells **(Figure 9.11)** (Gainetdinov et al., 1998; Nutt et al., 2004). Rebamipide patches increased DAT levels (40%) up to similar extent as rebamipide-oral administration (45%) against 6-OHDA-induced toxicity. Control and sham groups were found to be significantly indifferent.

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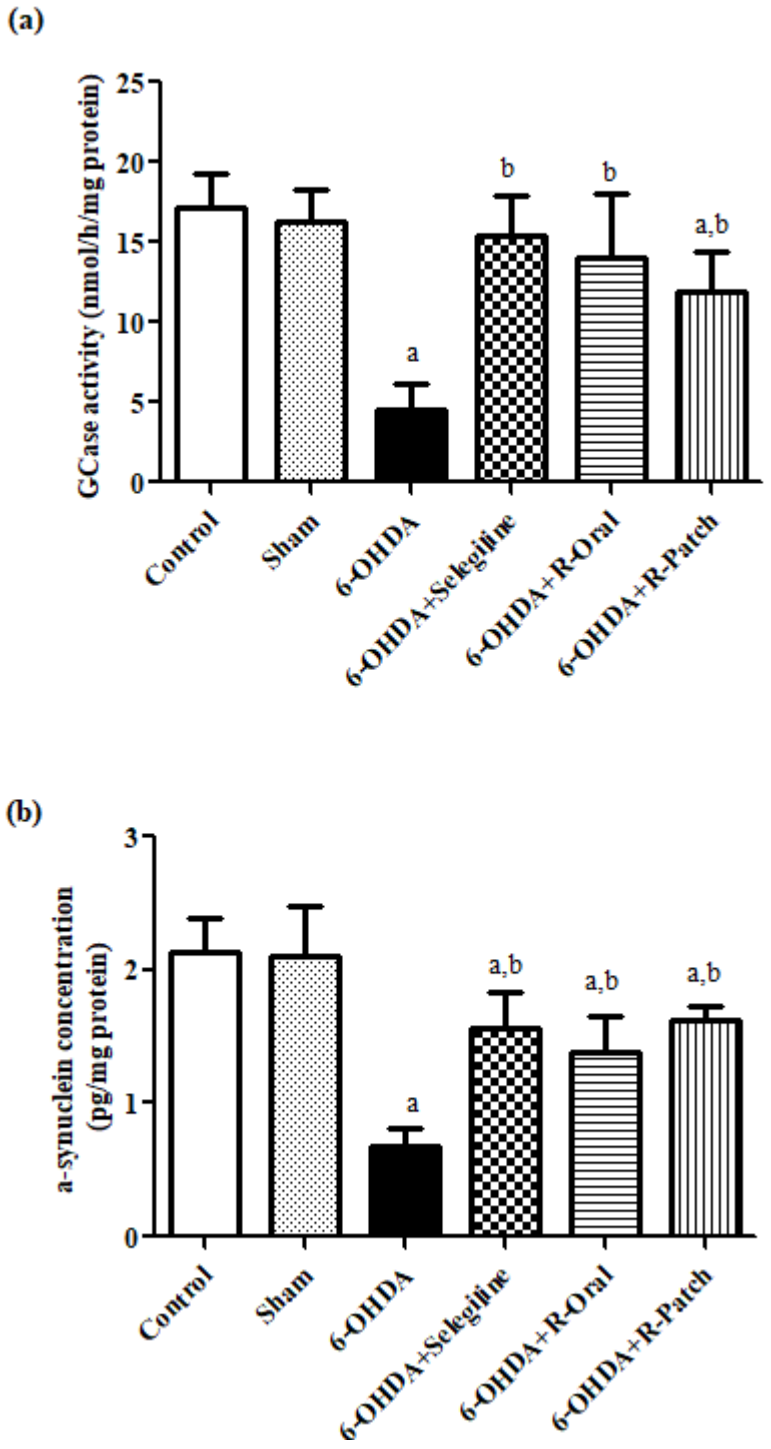

Figure 9.10 Effect of rebamipide (transdermal patches and oral) on 6-OHDA-induced alterations in GCase enzymatic activity (a) and soluble α-synuclein protein concentration (b) in ipsilateral nigral tissues of rats. All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, and $^{b}p < 0.05$ compared to 6-OHDA [One-way ANOVA followed by Student Newman–Keuls Post-hoc test]**.**

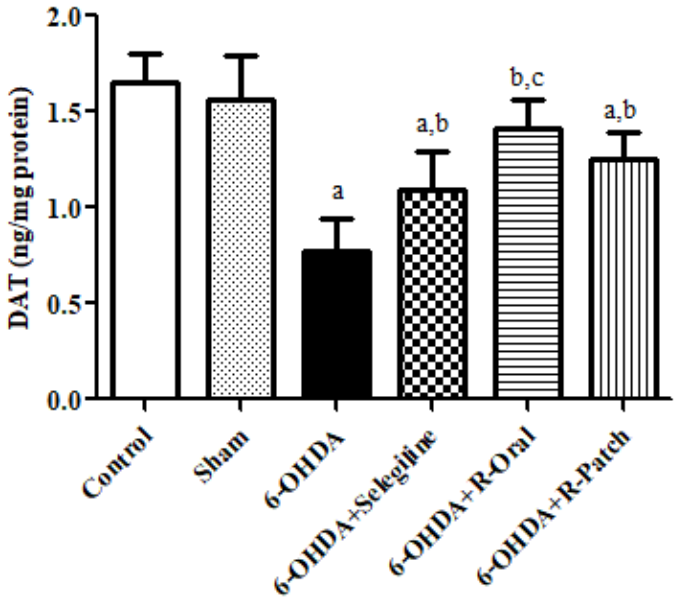

Figure 9.11 Effect of rebamipide (transdermal patches and oral) on 6-OHDA-mediated loss of DAT levels in ipsilateral striatal tissues of rats. All values are mean ± SD; n = 6;[a]$p < 0.05$ compared to sham, [b]$p < 0.05$ compared to 6-OHDA and [c]$p < 0.05$ compared to 6-OHDA+Selegiline [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

9.3.6.5. Quantification of rebamipide (HPLC analysis)

Rebamipide concentration was measured in plasma and CSF at the last day of experimental protocol in both the R-Oral and R-Patch groups. As analyzed by unpaired t test, no significant difference was observed between both the groups, neither in plasma nor CSF **(Table 9.7)**. This suggests that concentration of rebamipide obtained in plasma by applying 4 mg rebamipide-containing transdermal patches once a day was same as obtained by giving 80 mg/kg rebamipide oral twice a day. Same is the case with drug concentration in CSF also, suggesting the equal efficacy of patches and oral dose, along with high potency of patches.

Table 9.7 Plasma and CSF concentration of rebamipide

S.No.	Groups	Plasma Concentration (ng/mL)	CSF Concentration (ng/mL)
1	6-OHDA+R-Oral	413.95 ± 68.40	339.60 ± 131.02
2	6-OHDA+R-Patch	417.62 ± 68.74	354.05 ± 97.77

All values are mean ± SD; n = 6.

9.3.6.6. Transdermal patches of rebamipide attenuated 6-OHDA-induced reduction in the number of Nissl bodies in nigral tissues of rats

Significant differences in the percentages of Nissl bodies were observed in nigral tissues among groups [$F(5, 30) = 67.72$; $p < 0.05$] by using one-way ANOVA. In the present study, 6-OHDA decreased Nissl bodies up to 68% compared to control group **(Figure 9.12)**. No significant difference was found between control and sham groups. Transdermal patches of rebamipide increased the number of Nissl bodies up to 50% compared to 6-OHDA group, indicating the efficacy of transdermal patches. Rebamipide-oral groups also increased the number of Nissl bodies up to 54% compared to 6-OHDA group which was not found to be significantly different than rebamipide-patch groups. It shows that both the patches and oral dose showed same pharmacological efficacy. However, patches were found to be more potent.

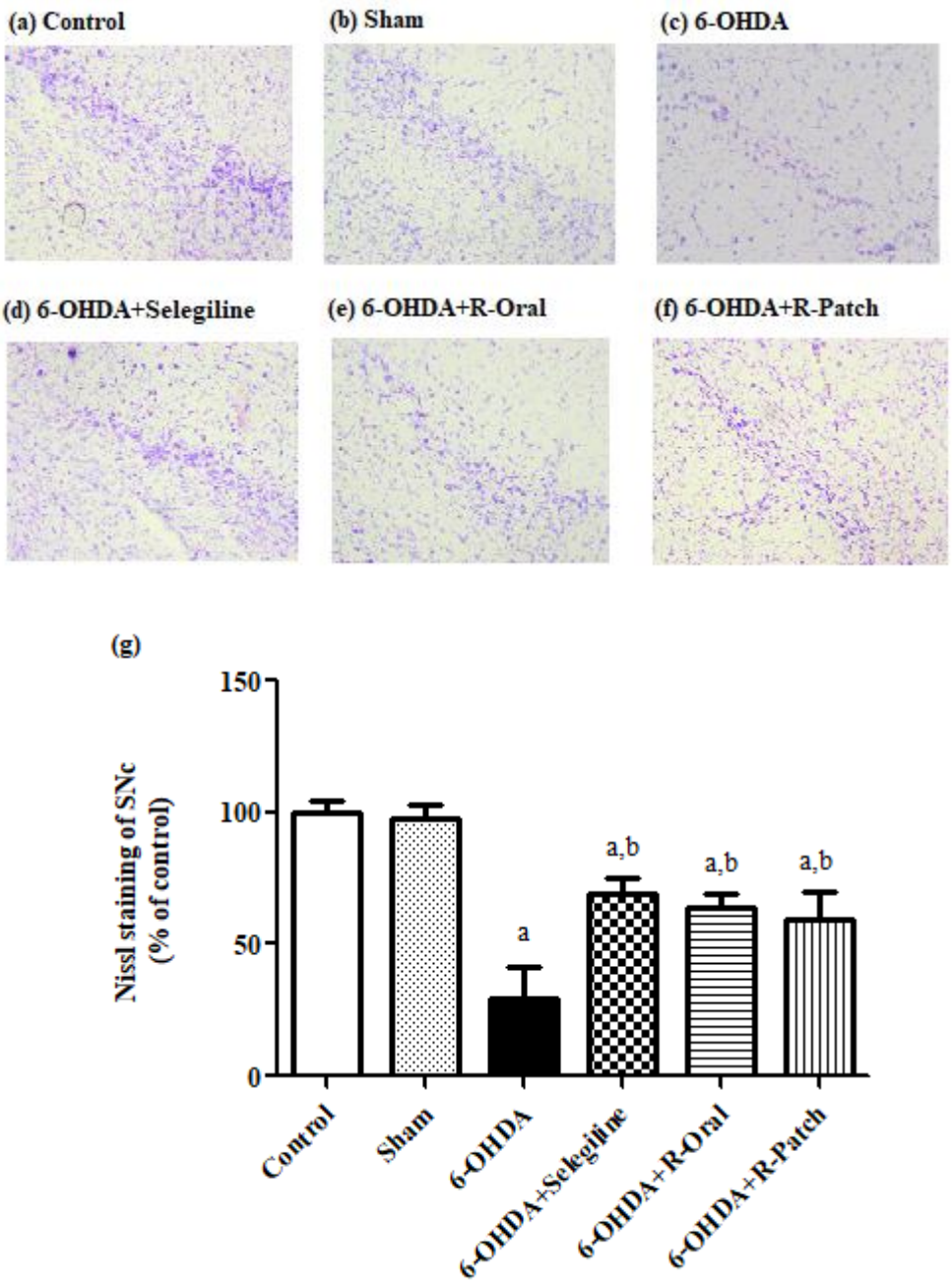

Figure 9.12 Nissl's staining of SNc in rats. Control (a); Sham (b); 6-OHDA (c); 6OHDA+Selegiline (d); 6-OHDA+R-Oral (e); 6-OHDA+R-Patch (f); Data of counting cells (g). All values are mean ± SD; n = 6; $^{a}p < 0.05$ compared to sham, and $^{b}p < 0.05$ compared to 6-OHDA [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

9.4. Discussion

The present study showed for the first time the preparation of rebamipide - loaded transdermal patches containing varying ratio of polymer and permeation enhancers, and their efficacy against PD model in rats. The novel feature of the present study includes the composition of the patch. Additionally, the study highlights that the effect of high amount of oral dose of rebamipide (80mg/kg twice a day) is equivalent to comparatively very low dose of drug in transdermal patches (4 mg drug/patch daily) against 6-OHDA toxicity. It indicates that both the patches and oral dose had same pharmacological efficacy. However, patches were found to be more potent than oral dose, thereby reducing the need of high dose and frequency for desired effects which makes the patches economical with high patient compliance. Transdermal patches of rebamipide attenuated motor deficits, α-synuclein pathology along with the deficiency of DA, GCase and dopaminergic cell markers TH and DAT against 6-OHDA-induced hemiparkinson's rat model. It also inhibited 6-OHDA-induced loss of Nissl bodies in nigral tissues of rats, indicating the actions of rebamipide-loaded transdermal patches against 6-OHDA toxicity to the same extent as oral dose. The drug concentration in plasma and CSF was also found to be similar for oral and patch groups.

Transdermal route is mostly preferred with high patient compliance rate over oral due to various applications, such as to avoid swallow dysfunction, nausea/vomiting, independence of meals, sleeping patient, less caregiving efforts, high absorption (Sieb et al., 2015). The compliance is also increased in older patients having chronic conditions, such as PD and Alzheimer's disease (Farlow and Somogyi, 2011). In the present study, transdermal patches of rebamipide were prepared by solvent casting technique to administer once a day. Solvent casting method was

selected due to its economically sound nature and good content uniformity (Malaiya et al., 2018). Four types of formulations were prepared by using varying concentrations of polymers and permeation enhancers. The patches were evaluated in terms of thickness, folding endurance, surface pH, weight, swelling and moisture loss. Alkaline or acidic pH may lead to irritation to the skin mucous membrane, which may also affect the degree of polymer-hydration, followed by skin adhesion. Therefore, the surface pH of the prepared patches was measured in order to optimize the drug permeation and skin adhesion (Malaiya et al., 2018). The surface pH was found to be within the range of skin pH, avoiding the possibility of any skin irritation when applied. The drug content in patches with minimum SD values indicates the uniform dispersion of drug throughout the patch. The patches were found to be uniform with low intra-batch variability in physicochemical characteristics, suggesting that the employed method to prepare the transdermal patches was reproducible with good quality. The *ex vivo* permeability was performed for all the formulations and found to be satisfactory. However, nearly complete permeation (91%) was achieved in 24h for formulation C probably due to presence of multiple permeation enhancers, high volume of plasticizer and low amount of polymer. Therefore, formulation C was selected for further characterization and *in vivo* studies. Surface morphology indicates that the patches were obtained as homogenous film with smooth surface. Due to absence of any significant shifting in the FTIR spectra of formulation C compared to the pure drug, it is proved that the purity of rebamipide was maintained in the patches without any interaction between drug polymers. The patches were found to be skin-compatible and no irritation and allergy was observed.

Motor deficits, such as tremor, bradykinesia, gait, muscular rigidity and loss of grip strength are observed in PD patients (Carrozzino et al., 2018; Vervoort et al., 2016). For *in vivo* studies, behavioral parameters include grip strength, apomorphine-induced head rotation, catalepsy and rotarod tests were performed on rats. 6-OHDA intrastriatal injection caused motor deficits, which were attenuated in a progressive manner by the daily administration of rebamipide-loaded transdermal patches up to the same extent as rebamipide oral administration, indicating the efficacy of transdermal patches against 6-OHDA toxicity. Motor deficits results due to the loss of DA in nigrostriatal region (Rodriguez-Oroz et al., 2009), 6-OHDA caused the reduction in striatal DA content which was increased by rebamipide patches, suggesting its DA protective effect. TH plays a major role in DA biosynthesis (Haavik and Toska, 1998; Zhu et al., 2012). 6-OHDA-induced TH-deficiency was attenuated by rebamipide patches, indicating protection of dopaminergic cell (Voutilainen et al., 2017). DAT, a determinant of extracellular DA concentration and specific for dopaminergic neurons is observed to be reduced in PD patients (Gainetdinov et al., 1998; Nutt et al., 2004). Rebamipide patches also increased striatal DAT levels in 6-OHDA-infused rats, indicating viability of dopaminergic cells. Another factors involved in the pathogenesis of PD includes GCase enzymatic deficiency and α-synuclein pathology (Di Maio et al., 2016). GCase is reported to be deficient in the brains of PD patients (Gegg et al., 2012; Migdalska-Richards and Schapira, 2016) and cause the reduction of α-synuclein oligomeric aggregates (Cleeter et al., 2013). In the present study, 6-OHDA-induced decrease in water-soluble concentration of α-synuclein indicates the accumulation of α-synuclein oligomeric aggregates as reported previously (Budi et al., 2012; Gu et al., 2016; Mishra et al., 2018). 6-OHDA also

caused deficiency in GCase enzymatic activity in nigral tissues of rats, as reported in Chapter 4 (Mishra et al., 2018). Both the GCase deficiency and α-synuclein pathology is inhibited by the administration of rebamipide-loaded transdermal patches in 6-OHDA-infused rats. Nissl's staining in SNc indicates the density of dopaminergic neurons (Domesick et al., 1983; Zaitone et al., 2012), therefore, rebamipide patches-induced increase in numbers of Nissl - positive cells against 6-OHDA toxicity denotes the high numbers of dopaminergic neurons in SNc tissues, as discussed in previous chapters. The stereological assessment of TH neurons evaluated by counterstaining with Nissl will be important. However, this serves as the limitation of the present study. The protective effects of transdermal patches of rebamipide were found to be similar to rebamipide oral administration against 6-OHDA toxicity. Rebamipide concentration was also found to be similar in plasma of both the R-Oral and R-Patch groups. Similarly, drug concentration in CSF was also same for both the groups. This proves that rebamipide-loaded transdermal patches having low drug dose are effective against 6-OHDA toxicity and the same is equivalent to high oral dose of rebamipide.

9.5. Conclusions

The results suggest that transdermal delivery of rebamipide have potential applications in PD therapeutics offering advantages in terms of decreased dose, non-invasive characteristics, low dosing frequency and simple termination of therapy. Transdermal patches prepeared using varying ratio of permeation enhancers and polymer were found to be uniform in terms of physicochemical characteristics. The formulation with highest *ex vivo* permeation did not show any interaction between polymers. The smooth surface and non-allergic profile of the transdermal patches

made them skin - compatible. Patches increased DAT and TH, the constituents of intact dopaminergic system, and also enhanced striatal DA content and GCase enzymatic activity along with reduction in α-synuclein pathology, all of which are involved in the regulation of PD pathogenesis. Total numbers of Nissl-positive cells were also increased. This leads to recovery of the motor functions in rats. The concentration of rebamipide in plasma and CSF was found to be same in both the patches and rebamipide-oral groups. The animal study proved that desired effects of rebamipide against 6-OHDA toxicity can be obtained with low-dose when given through transdermal route compared with oral administration. Both showed similar pharmacological efficacy, but patches were found to be more potent compared to oral dose. Overall, the present results support the potential of rebamipide-loaded transdermal patches against 6-OHDA toxicity, as depicted in **Figure 9.13**. Additionally, the patches are advantageous over oral route in terms of economical treatment, high patient compliance, low dose and decreased dosing frequency.

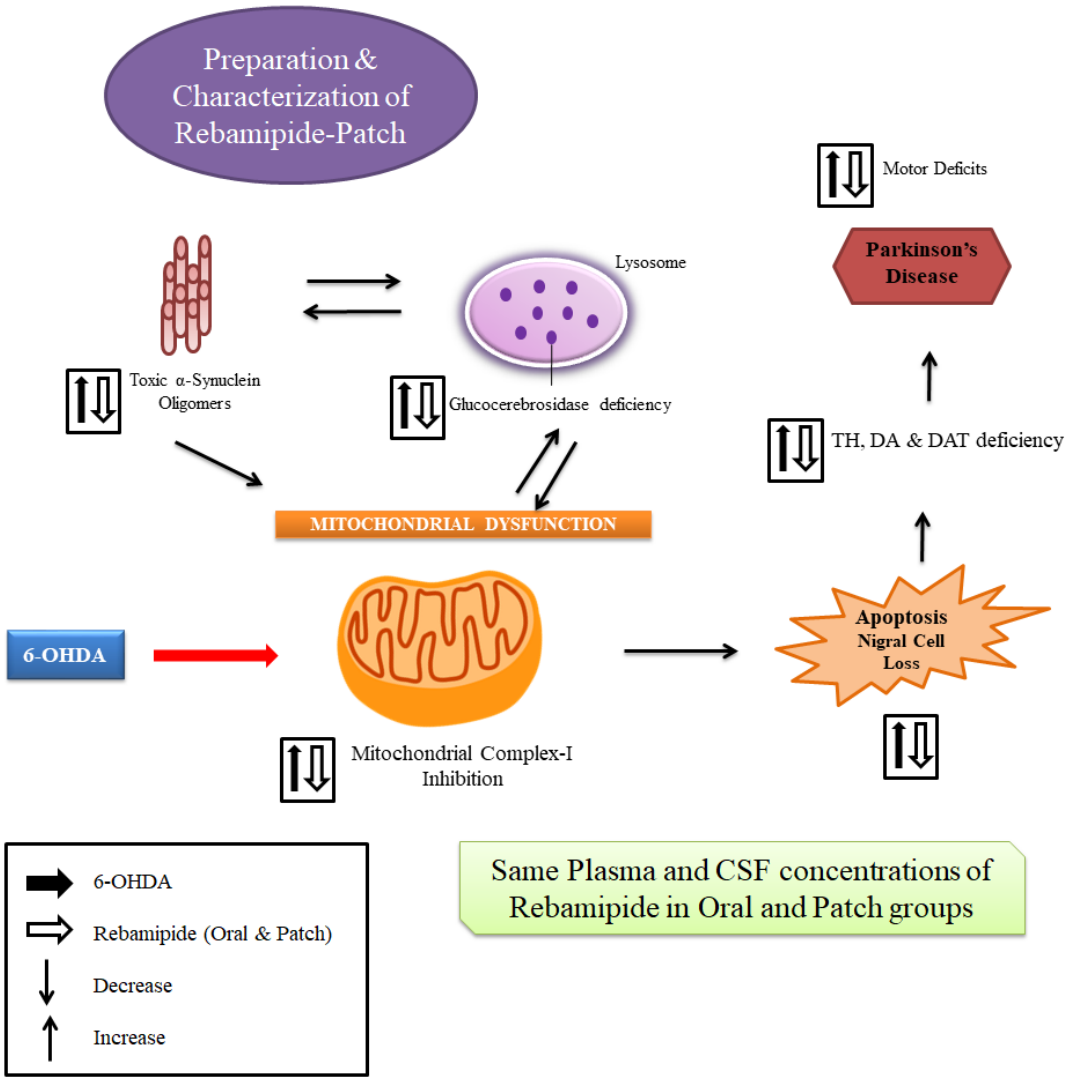

Figure 9.13 The outcome of specific objective for the design, characterization and evaluation of transdermal patches of rebamipide in rodent model of PD. Rebamipide-containing transdermal patches are formulated and observed to be uniform in physicochemical characteristics. Transdermal patches showing best *ex vivo* release profile are evaluated against 6-OHDA toxicity to compare their potency with oral dose. Rebamipide-containing patches inhibit mitochondrial dysfunction and α-synuclein pathology, which is followed by increase in GCase activity, number of Nissl bodies, levels of TH, DA and DAT along with recovery of motor behavior. The effects are found to be similar to oral rebamipide along with the same drug concentration in plasma and CSF of both the groups, indicating equal efficacy and high potency of drug-containing patches.