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Original Article

DESIGN, CHARACTERIZATION AND EVALUATION OF A NOVEL DOSAGE FORM OF REBAMIPIDE AS TRANSDERMAL PATCHES IN RODENT MODEL OF PARKINSON'S DISEASE

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ABSTRACT

Rebamipide at 80 mg/kg oral twice daily was previously found effective against a hemiparkinson's model in rats. However, the high dose and frequency limit its advantages. Therefore, for the first time, a novel dosage form of rebamipide as transdermal patches was formulated, characterized and evaluated againt a rodent model of Parkinson's disease. Four different transdermal formulations containing rebamipide were prepared using the solvent casting method (4 mg rebamipide/patch). Transdermal patches were observed to be uniform in terms of physicochemical characteristics. Rebamipide was administered through both the oral (80 mg/kg twice daily) and transdermal route (one patch daily) from Day-4 to Day-27 after unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA) in male rats. Patches increased levels of dopamine, dopamine transporters, tyrosine hydroxylase, and glucocerebrosidase enzymatic activity and decreased α-synuclein pathology and motor deficits in 6-OHDA-infused rats. All the animal-related parameters include 6 rats per group, and behavioral studies include 12 rats per group. Repeated measures of two-way ANOVA was used for the analysis of behavioral parameters and *ex vivo* permeability. Remaining *in vivo* parameters, physicochemical characteristics (weight, thickness, folding endurance, surface pH, swelling, moisture loss and drug content uniformity) and skin irritation studies were analyzed by one-way ANOVA. Rebamipide concentration using HPLC was analyzed by an unpaired t test. The effects of rebamipide patches and oral groups against 6-OHDA toxicity were similar and the concentration of rebamipide in plasma and cerebrospinal fluid of both groups was also observed to be the same. These preclinical findings suggest that a low rebamipide dose administered once daily through a newly-prepared transdermal patch (4 mg drug/patch) showed similar potential as a high oral rebamipide dose (80 mg/kg) administered twice daily in rats against 6-OHDA toxicity. Further studies including detailed pharmacokinetic and mechanistic data are required in order to translate the information successfully from animal to clinical studies.

KEYWORDS: Rebamipide; Transdermal patches; 6-Hydroxydopamine; Parkinson's disease; Glucocerebrosidase

1. Introduction

The drug rebamipide is clinically approved and has been being used for the treatment of gastritis, gastro duodenal ulcers and peptic ulcers from a very long time in Asian Countries as 100 mg tablets three times a day [1, 2]. Rebamipide at 80 mg/kg oral twice daily (at every 12 hour) was found to be effective against 6-OHDA-induced Parkinson's disease (PD) model in rats [3]. PD, one of the most widely spread neurodegenerative disorders is a fatal and several drugs are being studied for their

neuroprotective efficacy in PD [4]. Rebamipide is poorly absorbable and its plasma protein binding is high (98.4-98.6%) [5]. After oral administration once, only 71% and 41% radioactivity of 14C-labeled rebamipide was observed in the brain of rats when compared to blood and plasma levels [6]. The low permeability and low aqueous solubility may be responsible because according to the USFDA (United States Food & Drug Administration), rebamipide is classified as BCS (Biopharmaceutics Classification System) Class-IV agent [7, 8]. Solubility is the most important rate-limiting parameter for orally administered drugs to achieve the required bioavailability and elicit pharmacological response [9]. Lipid-made cell surface, such as blood brain barrier is penetrated by lipophilic drugs rapidly compared to hydrophilic drugs [10], whereas hydrophilic drugs are absorbed faster through the gastrointestinal membrane [11]. The absorption is found to be slower for drugs having poor water solubility which is responsible for their inadequate bioavailability [9]. Rebamipide is highly lipophilic and hydrophobic drug [8, 12], due to which it may not be well absorbed over gastrointestinal membrane through passive diffusion [11, 13], but is able to permeate the blood brain barrier [14]. This is the reason that the drug has low oral bioavailability (4.8 ± 1.4%) [15] and it is found to be neuroprotective in PD, but only in high doses (80 mg/kg oral. twice a day) [3]. Therefore, the prime objective of the present study is to prepare such a formulation which can decrease the required dose and frequency of rebamipide against PD model in rats.

Among the newer methodologies for administration of drugs is the use of transdermal approaches. Transdermal drug delivery has advantage 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 [16]. Transdermal patches have been designed as the therapeutics of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease [16, 17]. Drug is loaded inside a patch in a relatively high dosage and the patch is directly applied on the skin for the relatively longer period. Skin consists of epidermis (100-150 µm thick) as outer layer with no blood flow. The outermost layer of epidermis, namely stratum corneum, plays important role in transdermal drug delivery. Dermis is present beneath the epidermis and contains group of capillaries for blood transportation throughout the body. Therefore, drugs having ability to penetrate stratum corneum, may reach blood stream directly through passive diffusion process [18]. Stratum corneum layer of epidermis is chemically inert, but viable epidermal layers may display enzymatic activity to some extent, indicating not much loss of drug during absorption [19, 20]. The diffusion of drug will be continued into the blood for longer duration due to higher concentration of drug in the patch compared to blood, maintaining constant drug concentration in blood flow [18]. Currently, several drugs are available as transdermal patch systems for neurologic and other disorders in adults, including rotigotine, lidocaine, rivastigmine, diclofenac and capsaicin [21]. 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 [22]. Topical rotigotine is also reported to improve locomotor activity upto 48 hrs 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 upto 2 hrs [23]. Therefore, transdermal route is helpful in reducing the dose frequency as required in present study.

Drugs having poor oral absorption, short half-life and low tolerability of the oral formulation may be administered through transdermal delivery [21]. 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) [24, 25]; molecular weight should be < 600 dalton (rebamipide = 370.786 dalton) [26]; lipophilicity, an important parameter for transdermal administration because lipid solubility is responsible for systemic absorption of topically-administered drugs [27]; half-life should be 10 h or less (rebamipide=7.48 ± 0.92 h) [25, 28]; low oral bioavailability (rebamipide = 4.8 ± 1.4%) [15, 25]; hydrophobic drug with aqueous solubility >1mg/mL (water-solubility of rebamipide = 0.03 mg/mL) [29, 30]. Having these properties except aqueous solubility range, rebamipide is an ideal candidate for transdermal administration. Many drugs with low aqueous solubility (<1 mg/ml) including furosemide have been successfully developed into transdermal formulations by using suitable solubilizers and permeation enhancers [31]. Polyethylene glycol 400 and propylene glycol were also reported to increase solubility of rebamipide [32]. Therefore, if given through transdermal route and by using suitable permeation enhancers and solubilizers, the desired pharmacological effects of rebamipide may be obtained with low doses [compared to high oral dose (80 mg/kg twice a day)]. Hence, novel dosage form of rebamipide was prepared. Considering the pharmacotherapeutic benefit of rebamipide, various formulations have been prepared earlier in order to enhance its oral bioavailability which include but not limited to solid dispersion system, nanocrystal tablets, lipid nanoemulsions, bilayer tablet consisting of nanosuspension and colloidal nanoparticles [8, 33-36]. However, nothing reaches to clinical picture yet. Formulating a transdermal patch for rebamipide introduces a novel route tailored to enhance therapeutic consistency, maintain steady plasma levels, and improve patient adherence- especially in long term therapies. Therefore, rebamipide-loaded transdermal patches were prepared for the first time using different polymers and permeation enhancers. These were 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.

2. Materials and methods

2.1. Animals

If necessary, subsections must be used [2]. Adult albino male rats (250-300 g) of Charles-Foster strain were procured from Central Animal House, Institute of Medical Sciences, Banaras Hindu University. All the experiments performed during the light phase (9:00 and 16:00 h) on the basis of guidelines of National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Experimental

protocols were approved by Institutional animal ethical committee, Banaras Hindu University (Dean/2016/CAEC/33; dated 7th May, 2016).

2.2. Materials

Rebamipide was obtained as a gift sample from Akums Drugs & Pharmaceuticals Ltd., New Delhi, India. The reagents used were of analytical and high-performance liquid chromatography (HPLC) grades.

2.3. Preparation of 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. Backing membrane was prepared using aqueous solution of polyvinyl alcohol (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 minutes to attain the homogenous solution without bubble-formation. Homogenous solution (5 mL) was poured into the surface of petridish covered with aluminium 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 [37].

2.4. Formation of patches over the backing membrane

Rebamipide is classified as BCS Class-IV agent due to its low aqueous solubility and low permeability [7, 8], 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. Patches were prepared using solvent casting method [38]. HPMC-METHOCEL K100LV was used as polymer due to its suitability for low solubility-drugs [39]. 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 [37, 40]. The composition of patches is shown in **Table 1.** Weighed amount of drug [4.0 mg/patch] was dissolved in dimethylformamide (DMF, penetration enhancer) [41] and added in the beaker, followed by isopropyl myristate (IPM, permeation enhancer) [42]. PEG400 (solubilizer, plasticizer, penetration enhancer) [32, 43, 44] and sodium lauryl sulphate (penetration enhancer) [45] were added, followed by propylene glycol (PG; plasticizer and penetration enhancer) [43, 46]. Tween 80 (surfactant) [47] and transcutol (solubilizing agent, penetration enhancer) [43, 48] were added to the mixture. The contents were continuously stirred with magnetic stirrer for 20 minutes in order to get homogenous mixture. The homogenous mixture was casted on the prepared backing membrane placed in petridish, and allowed to dry for 24 h at room temperature [37, 46] . 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.

2.5. Physicochemical evaluation of transdermal patches

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. Three patches were taken randomly from each formulation to calculate the weight, thickness, folding endurance, surface pH, drug content uniformity, swelling behavior and percent moisture loss [49-53]. Results were shown as mean ± SD. 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 [51]. Surface morphology of patches was visualized using scanning electron microscope (SEM, EVO LS 10, Carl Zeiss, Germany) at an acceleration voltage of 10 kV.

2.6. Ex vivo drug permeation studies

Drug permeation studies were carried out using the skin of adult albino male rats of Charles-Foster strain. 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 6 h. The skin was shaved carefully and hairs were removed. Drug permeation studies were performed using Franz diffusion cells [52]. Drug-containing transdermal patches were kept adhered to the stratum corneum of the skin in the donor compartment of diffusion cell. PEG400 - phosphate buffer (40% v/v, 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 $\pm$ 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 24 h) 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 [38].

2.7. **Skin irritation studies**

The study was performed by using healthy adult albino male rats of Charles-Foster strain (weighing 250-300 g, n = 6 in each group) by the observer blinded to the groups. Their dorsal abdominal skin was shaved carefully avoiding any peripheral damage at 24 h 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 patch (R-patch, rats were applied with drug-loaded patches).

Table 1. Formulation of Transdermal Patches

S.No	Formulation Code	Solvent [Ethanol:Water] [1:2]	DMF:IPM [1:9]	Tween 80	SLS	Transcutol	HPMC-METHOCEL K100LV	PEG400	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% [0.25mL]	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% [0.25mL]	5% [0.5 gm]	7.5% [0.75 mL]	55% [5.5 gm]	10% [1 mL]	10% [1 mL]

DMF: Dimethylformamide (Penetration enhancer); IPM: Isopropyl myristate (Permeation enhancer); HPMC: Hydroxypropyl Methylcellulose (Polymer); LV: Low Viscosity; PEG: Polyethylene Glycol (Plasticizer, Solubilizer, Penetration enhancer); PG: Propylene Glycol (Solubilizer, Plasticizer); SLS: Sodium lauryl sulphate (Penetration enhancer): Tween 80: Surfactant; Transcutol: Solubilizing agent, Penetration enhancer.

Patches were removed after 48 h using alcohol swab. Skin irritation was evaluated on the basis of scoring method [54]. 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) [55].

2.8. **In vivo studies**

2.8.1. 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 day-1 (D-1) into the left striataum (A/P +1.0, L/M +3.0, D/V -5.0 relative to the bregam and dura), except control and sham groups. 6-OHDA was prepared as 5 µg/µL solution in 0.2 mg/mL ascorbic acid-normal saline and 4 µL (20 µg 6-OHDA) was injected into rats. Ascorbic acid-normal saline solution (4 µL) was injected to the rats of sham group. Selegiline was given as 10 mg/kg orally and used as positive control in present study [3]. Rebamipide (80 mg/kg) was administered orally twice daily at every 12 hour. Rebamipide suspension was prepared in 0.5% carboxymethylcellulose (CMC). CMC (0.5%) was given to control group orally [3]. Once the motor deficits are observed from D-4, rats were administered with respective drugs upto D-27. 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 24 h 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 [56]. The oral bioavailability of rebamipide is reported to be 4.8% [15]. Therefore, daily absorbed oral dose of rebamipide will be ~8 mg/kg, when given 80 mg/kg orally twice daily, the transdermal patches were planned to be 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 stratum corneum layer of epidermis is chemically inert, but viable epidermal layers may display enzymatic activity to some extent, indicating not much loss of drug during absorption [19, 20]. Various solubility and penetration enhancers were added in the formulation to support maximum penetration across the skin. Also, 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 [18]. 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 upto D-27 after 6-OHDA intrastriatal infusion, by removing the previous patches with the help of alcohol swab. Behavioral studies were conducted every week. 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 intraperitoneal 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 [57]. On D-28, cerebrospinal fluid (CSF) was also collected. Plasma and CSF samples were extracted by taking sample aliquots (100 µL) in microcentrifuge 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 (in-vivo pharmacokinetics). All the animals were killed by cervical dislocation on D-28. Nigral and striatal tissues from ipsilateral hemispheres were microdissected [58] 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). Experimental design is depicted in **Fig 1**.

2.8.2. Behavioral parameters

Rotarod test, apomorphine-induced head rotation, bar catalepsy and grip strength tests were performed [59-65].

2.8.3. Measurement of dopamine (DA), tyrosine hydroxylase (TH), dopamine transporters (DAT) and soluble α-synuclein levels

Commercially available kits were used to measure the levels of TH and α-synuclein in ipsilateral nigral tissues and DAT along with DA in ipsilateral striatal tissues.

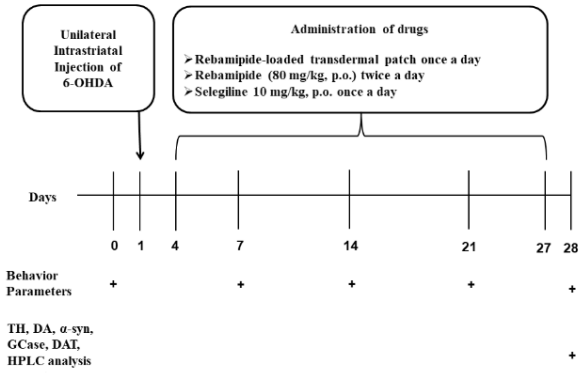

Fig. 1. Experimental Design for in vivo studies

2.8.4. Measurement of GCase activity

GCase activity was estimated in ipsilateral nigral tissues (~5 mg) of rat using earlier described protocol with slight modifications [66].

2.8.5. Quantification of Rebamipide (HPLC analysis)

The analyses were carried out using high-performance liquid chromatography (HPLC-Agilant 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 [67, 68]. The concentration of drug was calculated using standard curve [69].

2.9. Statistical Analysis

Repeated measures of two-way ANOVA was used for the analysis of behavioral parameters and ex vivo permeability. Bonferroni post hoc test was further utilized. 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. Results were denoted as mean + standard deviation (SD). $p < 0.05$ was considered significant throughout the analysis.

3. Results

3.1. Physicochemical characterization

3.1.1. Preformulation studies

As shown in absorption spectra of rebamipide (Fig 2), the λmax was obtained at wavelength of 230nm (Table 2). Calibration curve of rebamipide at λmax 230 nm is shown in Fig 3.

Table 2. Absorbance values of rebamipide solution at different wavelengths, obtained from absorption spectra (Fig.2)

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

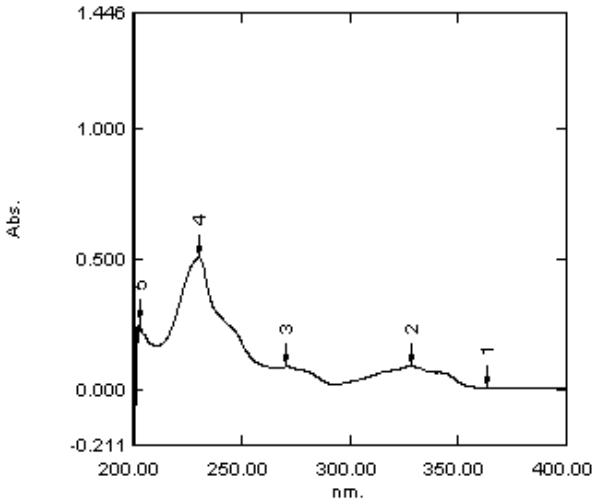

Fig. 2. Absorption spectra of rebamipide

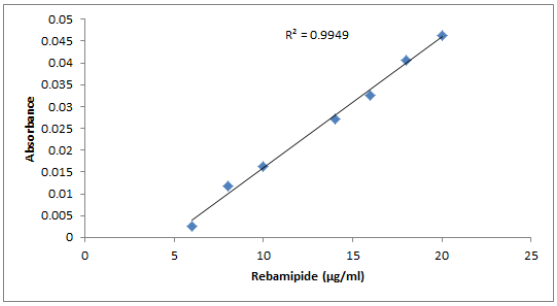

Fig. 3. Calibration curve of Rebamipide

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. All the patches were observed for their physical parameters and found to be smooth, flexible and uniform (Table 3). All the four types 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 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 [38]. 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.

Table 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.

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 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 [37]. 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 [37].

3.1.4. Moisture loss

Formulated patches were evaluated for stability under standard conditions. The results show 2.01 to 3.42% moisture loss. Formation C reflected lowest 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 4. All the patches are significantly different to each-other in terms of % moisture loss. However, formulations B and C which were prepared with high amount of PEG400 had significantly less moisture loss compared to the other patches. PEG400 acts as plasticizer, indicating the role of plasticizer behind the stability of formulation during long term storage.

Table 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 ± 1.41[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 ± 2.12[b,c]	3.04±0.07[a,b,c]

All values are mean + SD; n = 3; ap< 0.05 compared to formulation code A,bp< 0.05 compared to formulation code B, and cp< 0.05 compared to formulation code C [one-way ANOVA followed by post hoc Student Newman-Keuls test].

3.1.5. 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 [43, 46]. 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 (Fig 4). 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 upto 8h which is equivalent to half-life of rebamipide (7.48 ± 0.92 h) in rats [28]. However, the overall drug permeation release of formulation C at 24h was found to be significantly highest (91.46%; zero-order kinetics) 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 [56]. Therefore, the polymer was hydrated easily, swelled and results into faster and extended release of drug.

3.1.6. Fourier transform infrared (FTIR) spectroscopy

The pure drug in its FTIR spectrum showed characteristic sharp peaks at 1725.46 cm-1 due to aldehydes (C=O) stretch which confirms the intact aldehydic functionality of rebamipide. The same peak is observed in the patch also indicating no hydrogen bonding or any interaction strong enough to change the vibrational frequency.

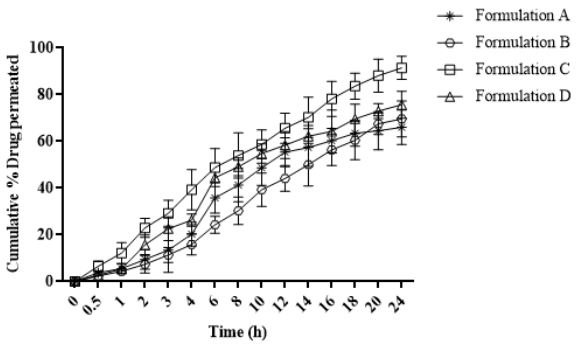

Fig 4. Ex vivo drug permeation profile of formulations (rebamipide-loaded transdermal patches) A, B, C and D through rat skin (mean ± SD; n = 3).

Another spectral peak at 1642.98 cm-1 due to C=O-NH stretching in pure drug has also been observed in the patch spectrum showing unaltered amide linkage of rebamipide and no significant interaction with polymer. Stretching vibrations were also observed due to primary and secondary amine at 1594.06 cm-1 and 1542.31-1505.13 cm-1 in pure drug. No significant peak shifts in the patch spectrum supports the conclusion that no bonding or significant interactions occurred between these groups and polymer matrix. Vibrations for –C-H rock alkanes and –C-H bend alkanes stretching were observed at 754.11 cm-1 (associated with long chain aliphatic compounds) and 1428.70 cm-1 (typical of methylene scissoring vibrations) respectively [Fig 5 (a)] for pure drug. Both these vibrations remain unchanged in position and shape in patch spectrum further confirm structural integrity of drug. Additionally, no novel significant peaks were observed in the drug-loaded transdermal patches [Fig 5 (b)], confirming the absence of any new chemical bond or no strong intermolecular interaction between the drug polymers. The characteristic peaks of rebamipide appeared with no significant shifting in the FTIR spectra of rebamipide-loaded transdermal patch, indicating drug stability. Overall, the presence of all major functional group peaks in the FTIR spectra of transdermal patch confirm that the molecular structure of rebamipide remains intact. This altogether validates that HPMC-METHOCEL K100LV - based transdermal patch is non-reactive which maintains the pharmacological properties of rebamipide.

3.1.7. Surface Morphology

Photomicrographs depict the SEM images of pure drug and patches at different magnifications (Fig 6). Elongated, plate- like crystals with sharp edges and uniform size distribution indicates well – defined crystalline structure of rebamipide (Fig 6a and b) which suggests its high purity and low amorphous content. The densely packed crystals may play important role in hindering the drug dissolution. However, emergence of smooth, continuous surface with loss of distinct crystals suggests homogenous film formation, good miscibility of drug and excipients which my play a significant role in increasing the permeability and long-term stability. Its amorphous nature may also enhance solubility and bioavailability of drug (Fig 6c and d).

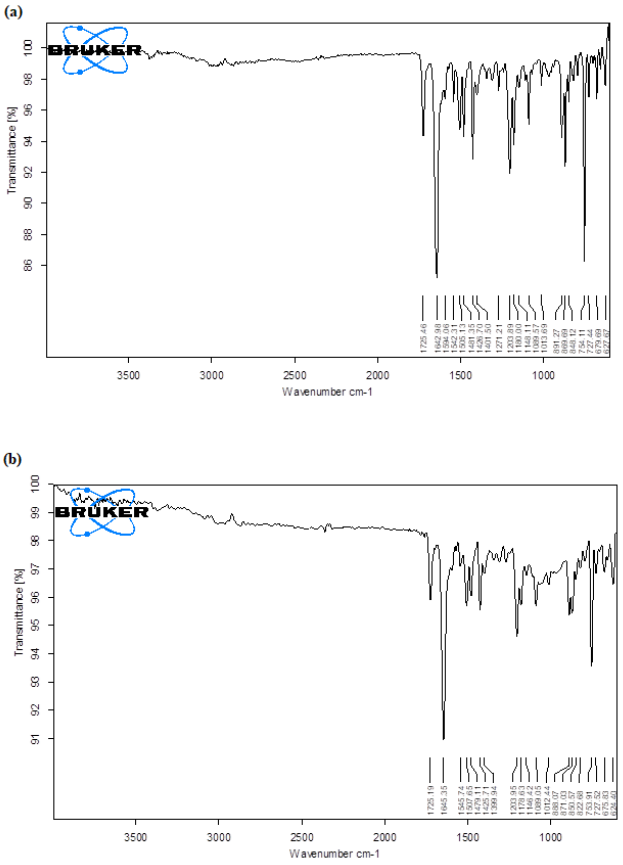

Fig 5. FTIR Spectra of pure drug rebamipide (a), and rebamipide-loaded transdermal patch formulation C (b).

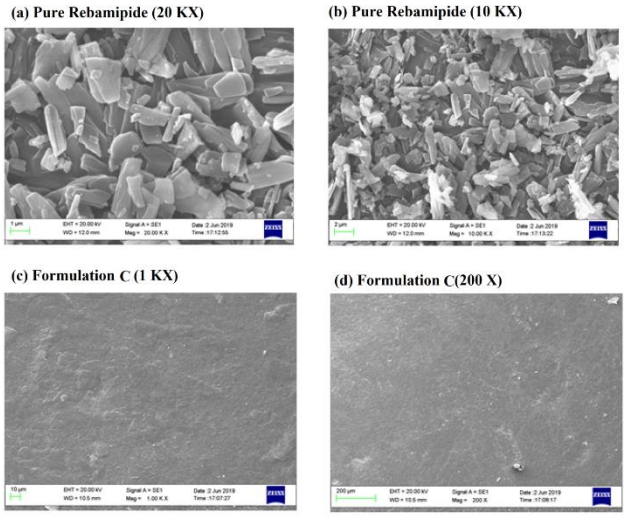

Fig 6. SEM images of pure rebamipide (a, b), and transdermal patches (formulation C) containing rebamipide (c, d) at different magnifications.

3.1.8. Skin irritation studies

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 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 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	0.24 ± 0.06
3	R-patch	0.94 ± 0.11^{a,b}
4	Formalin (0.8% v/v)	3.81 ± 0.41^{a,b,c}

All values are mean + SD; n = 6; ap< 0.05 compared to Control,bp< 0.05 compared to Adhesive tape USP type, and cp< 0.05 compared to R-patch[one-way ANOVA followed by post hoc Student Newman-Keuls test].

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

Motor deficits dominate the clinical picture of PD [70]. 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 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 (80 mg/kg twice daily) and rebamipide-patch (4 mg drug/patch daily) 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 (4 mg drug/patch daily) was not found to be significantly different than rebamipide-oral group (80 mg/kg twice daily), indicating high potency of patches against 6-OHDA toxicity for motor behavior.

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

Tyrosine hydroxylase (TH) is reported to be less in PD patients and takes part in the biosynthesis of DA [71]. 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 Fig 7 (a) and (b). No significant difference was observed between control and sham. TH and DA levels were significantly reduced (58% and 66% respectively) by 6-OHDA compared to sham group. Rebamipide both in oral (80 mg/kg twice daily) and transdermal (4 mg drug/patch daily) 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 (4 mg drug/patch daily) and oral dose (80 mg/kg twice daily) showed same pharmacological efficacy. Patches were more potent due to low amount of dose used.

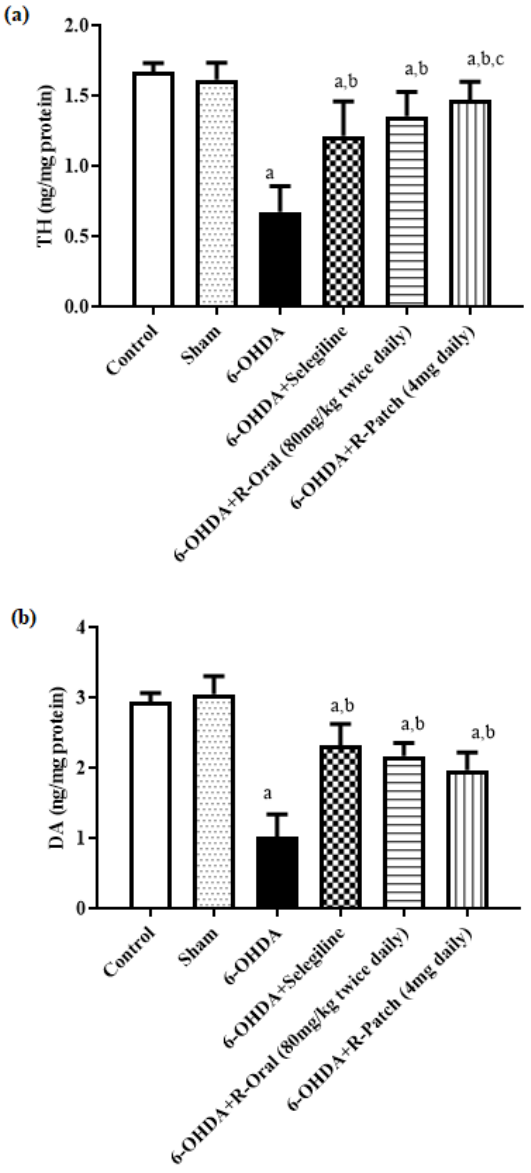

Fig 7. Effect of rebamipide (oral and transdermal patches) on 6-OHDA-mediated loss of TH (a) and DA levels (b) in ipsilateral nigral and striatal tissues of rats respectively. All values are mean + SD; n = 6; ^{a}p< 0.05 compared to sham, ^{b}p< 0.05 compared to 6-OHDA, and ^{c}p<0.05 compared to 6-OHDA+Selegiline [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

Table 6 Effects of rebamipide (oral and transdermal) on 6-OHDA-induced alterations in motor functions as assessed by apomorphine-induced rotations, latency in 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].

3.4. 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 Fig 8 (a) and 8 (b). 6-OHDA significantly decreased nigral GCase activity (72%) and soluble concentration of α-synuclein (67%) compared to sham groups. Rebamipide both in oral (80 mg/kg twice daily) and transdermal patches (4 mg drug/patch daily) increased GCase activity (68% and 62% respectively) and soluble α-synuclein concentration (51% and 58% respectively) upto similar extent against 6-OHDA group, suggesting high potency of patches.

3.5. 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 and imbalance in extracellular DA concentration (Fig 9) [72, 73]. Rebamipide patches (4 mg drug/patch daily) increased DAT levels (40%) upto similar extent as rebamipide-oral (80 mg/kg twice daily) administration (45%) against 6-OHDA-induced toxicity. Control and sham groups were found to be significantly

indifferent.

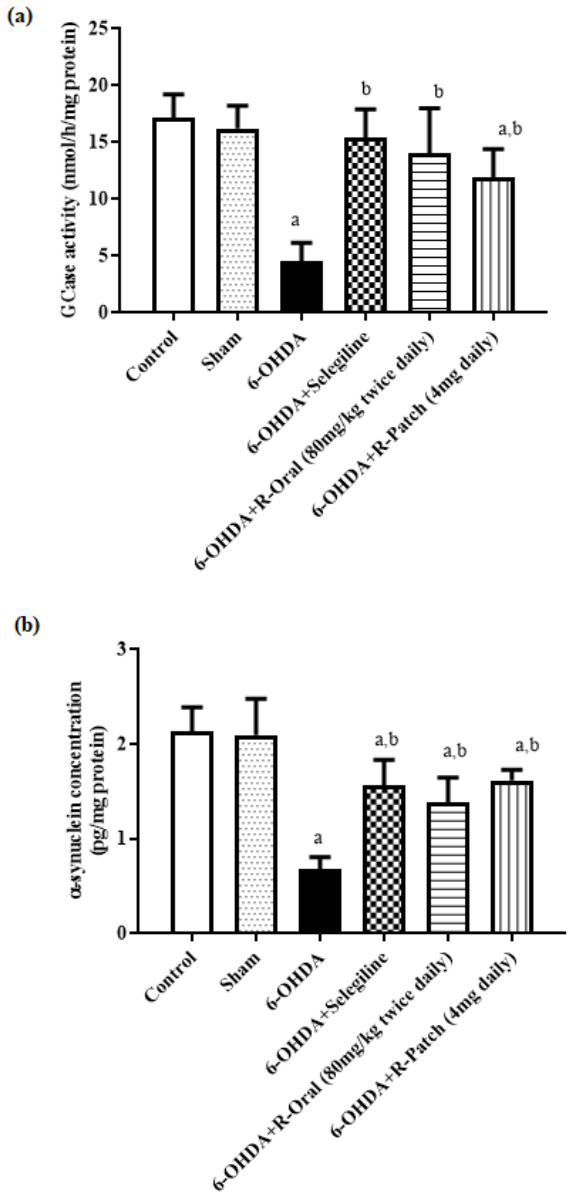

Fig 8. Effect of rebamipide (transdermal patches and oral) on 6-OHDA-induced alterations in GCase enzymatic activity (a) and soluble α-synuclein protein concentration (b) in ipsilateral nigral tissues of rats. All values are mean + SD; n = 6; ap< 0.05 compared to sham, and bp< 0.05 compared to 6-OHDA [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

3.6. 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 (80 mg/kg twice daily) and R-Patch (4 mg drug/patch daily) groups. As analyzed by unpaired t test, no significant difference was observed between both the groups, neither in plasma nor in CSF (Table 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 administering 80 mg/kg rebamipide oral twice a day. Same is the case with drug concentration in CSF also, suggesting the high potency of patches compared to oral dose. Drug quantification was carried out using a calibration curve exhibiting excellent linearity (R^2 = 0.9966). The method demonstrated consistent analytical response across the tested concentration range. All measurements were conducted under controlled and reproducible conditions. The representative chromatograms of plasma samples, and CSF samples have been included as supplementary figures (Figure S1).

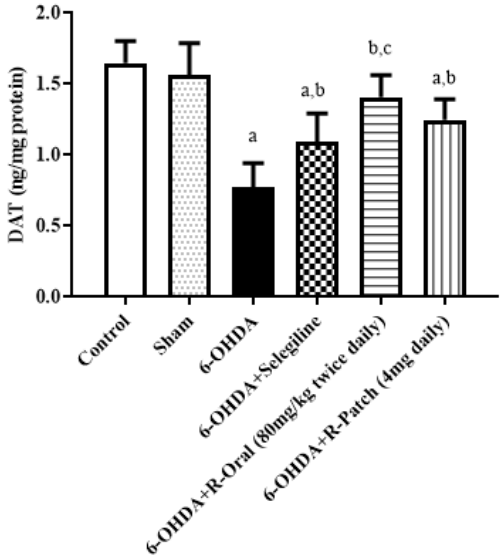

Fig 9. Effect of rebamipide (transdermal patches and oral) on 6-OHDA-mediated loss of DAT levels in ipsilateral striatal tissues of rats. All values are mean + SD; n = 6; ap< 0.05 compared to sham, bp< 0.05 compared to 6-OHDA and cp< 0.05 compared to 6-OHDA+Selegiline [One-way ANOVA followed by Student Newman-Keuls Post-hoc test].

Table 7 Plasma and CSF concentration of rebamipide

S.No.	Groups	Plasma Conc (ng/mL)	CSF Conc (ng/mL)
1	6-OHDA+R-Oral (80mg/kg twice daily)	413.95 ± 68.40	339.60 ± 131.02
2	6-OHDA+R-Patch (4 mg drug/patch daily)	417.62 ± 68.74	354.05 ± 97.77

All values are mean ± SD; n = 6.

4. Discussion

The present study showed the formulation of novel dosage form of rebamipide as transdermal patches along with its characterization and evaluation against 6-OHDA model of Parkinson's disease. For the first time, rebamipide - loaded transdermal patches were prepared containing varying ratio of polymer and permeation enhancers, and their high potency compared to oral dose against PD model in rats. The study fulfills the purpose of reducing the need of high dose and frequency of rebamipide for desired pharmacological effects against PD model in rats. The effect of high amount of oral dose of rebamipide (80mg/kg twice a day) is found equivalent to comparatively very low

dose of drug in transdermal patches (4 mg drug/patch daily) against 6-OHDA toxicity. It indicates that 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 along with high patient compliance. Transdermal patches of rebamipide (4 mg drug/patch daily) attenuated motor deficits, α-synuclein pathology along with the deficiency of DA, GCase and DA cell markers TH and DAT against 6-OHDA-induced hemiparkinson's rat model, indicating its high potent nature compared to oral dose. The drug concentration in plasma and CSF was also found to be similar for oral (80mg/kg twice a day) and patch groups (4 mg drug/patch daily).

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 [74]. The compliance is also increased in older patients having chronic conditions, such as PD and Alzheimer's disease [21]. 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 [37]. 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. The combination of substantial swelling (61-71%) and low moisture loss (2-3.4%) suggests that the patches may function as semi-occlusive systems with partial water vapor exchange and strong moisture retention which may favor sustained drug delivery while minimizing skin maceration. 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 [37]. 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, high permeation (91%) was achieved in 24 h 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 significant 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 dominate the clinical picture of PD [75, 76]. 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 (4 mg drug/patch daily) upto the same extent as rebamipide oral administration (80mg/kg twice a day), indicating the efficacy and potency of transdermal patches against 6-OHDA toxicity. Motor deficits results due to the loss of DA in nigrostriatal region [77], 6-OHDA caused the reduction in striatal DA content [3] which was increased by rebamipide patches, suggesting its DA protective effect. TH plays a major role in DA biosynthesis [71, 78]. 6-OHDA-induced TH-deficiency was attenuated by rebamipide patches, indicating protection of dopaminergic cell [79]. DAT, a determinant of extracellular DA concentration and specific for dopaminergic neurons is observed to be reduced in PD patients [72, 73]. 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 [80]. GCase, a lysosomal enzyme is reported to be deficient in the brains of PD patients [81, 82] and cause the reduction of α-synuclein oligomeric aggregates [83]. GCase deficiency cause the accumulation of its substrate glucocerebroside (GC) in lysosome which acts as scaffold for α-synuclein oligomers to develop [84]. α-synuclein in its monomeric form is found to be water-soluble. However during aging and toxic conditions, it turns water-insoluble and forms oligomers [85]. 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 [3, 85, 86]. 6-OHDA also caused deficiency in GCase enzymatic activity in nigral tissues of rats [3]. Both the GCase deficiency and α-synuclein pathology is inhibited by the administration of rebamipide-loaded transdermal patches in 6-OHDA-infused rats. The protective effects of transdermal patches of rebamipide (4 mg drug/patch daily) were found to be similar to rebamipide oral administration (80 mg/kg twice a day) 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 altogether proves that rebamipide-loaded transdermal patches having low drug dose (4 mg drug/patch daily) is effective against 6-OHDA induced toxicity and the same is equivalent to high oral dose of rebamipide (80 mg/kg twice a day).

5. Conclusions

The study introduces the preparation of novel dosage form of rebamipide as transdermal patches having varying ratios of polymers and permeation enhancers. The results suggest that transdermal delivery of rebamipide have potential applications in PD therapeutics offering advantages compared to oral dose in terms of decreased dose, non-invasive characteristics, economic nature, low dosing frequency and simple termination of therapy. The animals study proved that desired effects of rebamipide against 6-OHDA toxicity can be obtained with low drug dose when given through transdermal route compared to

oral administration. Both showed similar pharmacological efficacy but patches were found to be more potent than oral dose. Overall, our results support the efficacy and high potency of novel dosage form of rebamipide as transdermal patches against PD model which reduced the need of high dose and frequency of rebamipide for desired effects.

6. Limitations

The study consists of basic physiochemical properties, ex-vivo permeation studies as proof of concept and in vivo studies to observe the therapeutic effects of novel formulated rebamipide transdermal patches (4 mg drug/patch once daily) compared to oral rebamipide dose (80mg/kg twice daily) against 6-OHDA toxicity. Potential interactions between rebamipide and the excipients within the final transdermal formulation was assessed using FTIR to observe no ambiguity in peak shifts. However, small shifts in peak position, changes in intensity, or band broadening can also indicate interactions. Hence, exhaustive comparison of the full overlaying spectra (drug, polymer, physical mixture, and final patch is further required to conclude that there is no drug–polymer interactions. Detailed pharmacokinetic data including ADME and other parameters at different time points in preclinical settings in various animal species is further required considering the differences in pharmacokinetics between rats and humans. The study involves the stereotaxic injections of 6-OHDA to mimic PD condition in rats. Exhaustive mechanistic data is further needed in order to translate all the parameters successfully from animal to clinical studies and with real life PD patients.

Author Contributions: AM and SK conceptualized and designed the study. AM collected the data. SK and AM analyzed, interpreted, drafted and finalized the data for intellectual content and context. SK did the final approval and takes overall responsibility of the published work.

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Conflicts of Interest: AM and SK are inventors on an Indian patent application "Rebamipide Loaded Transdermal Patch For Neurodegenerative Disease" (201911039568, 30/Sep/2019).

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