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USE OF PRONISOME GEL IN ANTIDEPRESSANT THERAPY: DESIGN AND EVALUATION

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ABSTRACT

Selegiline is an irreversible monoamine oxidase (MAO)-B inhibitor used at low doses for the adjunctive treatment of Parkinson's disease. Mouth ulcers and stomatitis may occur with the oral lyophilisate. Selegiline is interacted with tyramine in food. Early studies suggested that oral selegiline was effective as an antidepressant in a dose range that preserved the selectivity of MAO-B inhibition. However further open trial studies have indicated that dose of oral selegiline required for clinical antidepressant like activity in most patients are relatively high (≥30 mg/day) and nonselective producing inhibition of MOA-A. Such a loss of specificity would mean that patients taking selegiline for depression would need to observe dietary restriction applicable to nonselective MAOIs. Unlike oral MAO inhibitors, transdermal selegiline delivers antidepressant drug concentrations to the central nervous system without substantially impairing gastrointestinal MAO-A activity. At the target dose of 6 mg per 24 hours, tyramine dietary restrictions are not needed. Dermal therapeutic formulation like proniosome gel of selegiline was developed and evaluated for effective treatment of depression. The present work deals with the formulation and characterization of selegiline hydrochloride Proniosome gel with an aim to enhance drug permeation through the barriers of skin and to maintain the controlled plasma level concentration. The optimized proniosomal gel P8 containing selegilne hydrochloride showed significant anti-depressant activity at $P < 0.05$, Primary Dermal Irritation Index (PDII) value of 0 and drug release of 92.41% over a period of 24 hrs. The results led to conclude that proniosomes offers an effective alternative colloidal carrier approach in transdermal drug delivery.

KEY WORDS: Selegiline hydrochloride, Spans, Tweens, antidepressant activity, in-vitro release and in-vivo studies.

INTRODUCTION

Drug delivery systems are used to ensure that the drugs get into the body and reach the area where they are needed. These systems must take a number of needs into account, ranging from ease of delivery to the effectiveness of the drug. [1] Recently several technical advancements have been made. They have resulted in the development of new techniques for drug delivery. Transdermal route of administration is one, wherein active ingredients are delivered across the skin for systemic distribution. Transdermal delivery systems, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first-pass metabolism and a higher compliance. [2] A continuous interest toward the dermal and transdermal products can be seen, offering several advantages. [3]

Niosomes represent an emerging class of novel vesicular systems. Niosomes are vesicles composed primarily of synthetic non-ionic surfactants and cholesterol having a bilayer structure. Niosomes are formed by hydration of non-ionic surfactant dried film resulting in imbibing or encapsulating the hydrating solution. [4, 5] Although, niosomes exhibit good chemical stability, they are physically less stable. Aqueous suspension of niosomes exhibit aggregation, fusion, leaking of entrapped drug thus reduces shelf life of dispersion. [6] Hence, 'dry niosomes' can be prepared which are often called as 'Proniosomes', which avoid many problems associated with niosomes. Proniosomes can be hydrated immediately before use to give niosomal dispersion. Proniosomes are dry, free flowing granular product which upon hydration gives a multilamellar niosomal dispersion. In addition convenience in transport, storage, and dosing makes proniosomes as a promising carrier. This proniosomal drug delivery has attracted towards transdermal delivery of drugs because surfactants themselves act as penetration enhancers and are biodegradable, non-toxic, amphiphilic, possess property of encapsulation and can entrap both hydrophilic as well as lipophilic drugs. [7] They are converted into niosomes respectively upon simple hydration or by the hydration of skin itself after application.

Depression is one of the most common psychiatric disorders. The Diagnostic and statistical Manual of Mental Disorders [DSM-IV-TR], categorizes post-stroke depression as a mood disorder due to a general medical condition. [8] MAO have been used to treat major depressive

disorder. Selegiline a preferential MAO type B inhibitor is currently used in the treatment of depression. Selegiline Hydrochloride has steady state half life 2 hrs, oral dose of 10 mg daily, oral bioavailability 4.4% and protein bonding of 94%. [9, 10] The present investigation deals with the formulation and characterization of selegiline hydrochloride Proniosome gel with an aim to enhance drug permeation through the barriers of skin and to maintain the controlled plasma level concentration.

MATERIALS AND METHODS

Materials

Selegiline hydrochloride was obtained as gift sample from Embio Limited, Mumbai. Span20, 40, 60, 80, Tween 20, 60 and cholesterol were procured from S. D. Fine chemicals Pvt. Ltd, Mumbai. Soya lecithin was procured from High Media lab, Mumbai. All other reagents used were of analytical grades.

Preparation of Proniosomal Gel: [11]

Proniosomal gel was prepared by a coacervation phase separation method Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.5 ml) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till a clear solution was formed which was converted into proniosomal gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization. [12] Compositions of proniosomal gel formulations are given in (Table 1).

Evaluation of proniosomal gel formulations

Surface morphological study: The morphology of niosomes derived from proniosome gel was studied using Scanning Electron Microcopy (SEM).

Scanning electron microscopy (SEM) study

Surface morphological study mainly is done with the help of scanning electron microscopy (SEM). Scanning electron micrograph for optimized formulation is shown in Fig. 8. which indicated that vesicles formed in proniosome gel formulation were spherical, rounded, smooth and there was no formation of any aggregates.

Vesicle Size analysis: Hydration of proniosome gel (100 mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 45 x magnification. The sizes of 200 - 300 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Rate of spontaneity: 10 to 20 mg of proniosome gel was transferred to the bottom of a clean stopper glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature, 2 ml of phosphate saline (0.154 M NaCl) was added carefully along the walls of the glass bottle and left in a test-tube stand. After 20 min, a drop of this saline solution was withdrawn and placed on Neubauers Chamber to count the number of vesicles. The number of niosomes eluted from proniosomes was counted. [13]

% Entrapment efficiency: To evaluate the loading capacity of proniosome systems proniosome gel (100 mg) was dispersed in distilled water and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 18000 rpm for 40 min at 5^{o}C. The clear fraction was used for the determination of free drug at 258 nm spectrophotometrically. The percentage entrapment efficiency was calculated from following equation.

% Entrapment efficiency = [Total drug- Free drug / Total drug] x 100

Table 1. Composition of Selegiline hydrochloride proniosome gel formulations.

Formulation code	Drug (mg)	Surfactant Weight (mg)		Lecithin (mg)	Cholesterol (mg)	Alcohol (ml)	pH 7.4 phosphate buffer (ml)
P1	10	Span 20 (S20)	180	180	20	0.25	0.16
P2	10	Span 40 (S40)	180	180	20	0.25	0.16
P3	10	Span 60 (S60)	180	180	20	0.25	0.16
P4	10	Span 80 (S80)	180	180	20	0.25	0.16
P5	10	Tween 2 0 (T20)	180	180	20	0.25	0.16
P6	10	Tween 40 (T40)	180	180	20	0.25	0.16
P7	10	Tween 60 (T60)	180	180	20	0.25	0.16
P8	10	Tween 80 (T80)	180	180	20	0.25	0.16

pH determination: The pH of each Proniosome gel was determined using pH meter. The electrode was first calibrated with pH 4.0 and pH 7.0 solutions, then sample readings were recorded on pH meter. [14]

In-vitro release studies: In-vitro release studies on proniosome gel were performed using Franz-diffusion cell. The capacity of receptor compartment was 75 ml. The area of donor compartment exposed to receptor compartment was 1.41cm^2. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosome gel was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 32±1^{o}C. [15] Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval i.e., every hour, 5 ml of sample was withdrawn and replaced with 5 ml fresh buffer to maintain sink condition. Samples withdrawn were analyzed spectrophotometrically at 258 nm.

In-vitro permeation studies

In-vitro permeation studies were carried out on optimized proniosome gel formulation using excised rat abdominal skin**.**

Preparation of the rat skin [16]

The experiment was conducted according to the protocol approved by the institutional animal ethics committee (IAEC). The experiment was conducted according to the guidelines of CPCSEA (Committee for the purpose of control and supervision of experiment on animal). The male albino rats weighing 170 -190 g were sacrificed by decapitation. The fresh abdominal skin was excised and separated along the epidermal junction. The hair of skin was removed using depilatories. The process of the removal of hair did not alter the skin properties. It was kept in water bath maintained at 60^{0}C for exactly 50 sec. The heat treated skin was cleared of it subcutaneous fatty substance and immediately kept in refrigerator at 10^{0}C. This step maintained integrity and viability of the skin.

Permeation studies

The permeation studies on optimized proniosome gel formulation were carried out by same procedure as used in in-vitro release studies, except for the cellophane membrane, the excised rat abdominal skin was used as a membrane.

In-vivo studies

Anti-depressent activity [17, 18]

Tail suspension test (TST) was employed for screening antidepressant activity in mice. The tail-suspension test is a mouse behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors. Mice of either sex weighing in between 20 - 25 gm were used for the study. Two Groups of 6 animals each were used as control and test. For control group plain gel (without drug) and for test group optimized proniosome gel, was applied to dorsal surface of mice 30 min prior to testing. After application of the gel mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape, placed approximately 10 cm from the tip of the tail. In such a position the mice cannot escape or hold on to nearby surfaces. During this test, typically 6 mins in duration, the resulting escape oriented behaviors (duration of immobility) are recorded and then subsequently analyzed. Mice were considered to be immobile in absence of body movement, hung passively, and were completely motionless for at least 1 min. The test was conducted in a dim lighted room and each mouse was used only once in the test.

Statistical analysis: The immobility time in tail suspension test (data) was analyzed using Graph Pad in stat by one way analysis of variance (ANOVA). Results were expressed as the means ± SEM. The level of statistical significance adopted was **P<0.01, when compared with the control group.

Skin irritation studies [19]

The skin irritation test was performed on six healthy albino rabbits of either sex weighing between 2.0 to 2.5 kg. Rabbits were procured from the central animal house of N.E.T. pharmacy college, Raichur (CPCSEA 15/10/2011). The execution of the project was effected with due approval of study protocol by the Institution Animal Ethics Committee (IAEC). The rabbits were depilated on left and right dorsal surfaces by approved techniques. Aqueous solution of formalin 0.8% v/v was used as standard irritant. Optimized Proniosome gel formulation was used as test. Formalin 0.8% v/v was applied on the left dorsal surface of each rabbit; whereas the proniosome gel was applied on identical site, on the right dorsal surface of the rabbit. The proniosome gel was removed after a period of 24 hrs with the help of alcohol swab. The skin response was examined and was scored using Draize Evaluation of Dermal Reactions for erythema and edema for each rabbit at the end of 24 hrs. The average

value was calculated which gave the Primary Dermal Irritation Index (PDII). The resulting Primary Dermal Irritation Index (PDII) was classified as follows

PDII	Classification
<0.5	Non-irritating
0.5 – 2.0	Slightly irritating
2.1 – 5.0	Moderately irritating
>5.0	Severely irritating

RESULTS AND DISCUSSION

Surface morphological studies revealed that proniosomes formed were spherical and homogeneous. Scanning electron micrograph for optimized formulation P8 (Fig. 1) indicated that vesicles formed were spherical, rounded, smooth and there was no formation of any aggregates. Determination of vesicle size is important for the topical application of vesicles. Brain et al demonstrated that vesicle more than 10 micrometer remains on skin surface and the vesicle of 3-10 micrometer concentrates in follicle and less than 3 micrometer penetrates the stratum corneum. [20] Results of vesicle size of Selegiline hydrochloride proniosomes (Table 2), indicated that vesicle formed with Span were smaller in size than vesicle formed with Tweens; this was due to greater hydrophobicity of Spans than Tweens. It was observed that increasing in hydrophobicity decreased surface energy of surfactants, resulting in smaller vesicle size. The vesicle size was found in the range of 2.85±1.3 µm to 5.71±1.28 µm. Rate of spontaneity is the number of niosomes formed after hydration of proniosomes for 20 mins. Rate of spontaneity for different proniosome gel formulations were varied between 6.28±0.18 mm^3×1000 (P8) to 9.86±0.52 mm^3×1000 (P4) (Table 2). Proniosome formulations prepared with Spans formed niosomes more spontaneously than Tweens. It was also observed that as the vesicle size decreased the rate of spontaneity increased. Entrapment efficiency was found to be higher in case of proniosomes prepared with Span40 and Span60 than proniosome prepared with Tween, this was due to the fact that Span are more hydrophobic than Tween, and act as solid at room temperature with higher phase transition temperature (Tc), low HLB value and long alkyl chain length. The results are shown in Table 2.

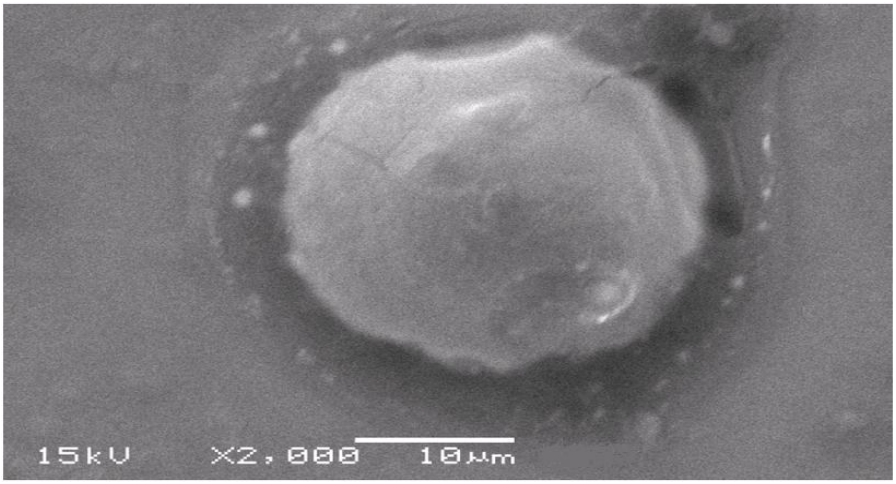


Fig. 1: Scanning electron microscopy (SEM) image of optimized Selegiline hydrochloride proniosome gel P8.

The pH was determined in order to investigate the possibility of any side effects in-vivo due to acidic or alkaline pH which may irritate the skin. The pH was found in between 5.2 (P1) to 6.3 (P8) which was well within the physiological skin surface pH [21] (Table 2). Changes in pH are reported to play an important role in the pathogenesis of skin diseases. Maintaining the skin pH helps maintain a proper balance of the "acid mantle" which aids in protecting the body from bacteria and helps prevent moisture loss. [22]

In vitro release studies

In vitro release studies are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its in vivo performance since drug release dictates the amount of drug available for absorption. The proniosome gels prepared with different Spans as surfactants i.e., P1(S20), P2 (S40), P3 (S60) and P4 (S80) released 61.25%, 65.62%, 68.95% and 67.0% of drug at the end of 24 hrs. The proniosome gels prepared with different Tweens as surfactants i.e., P5 (T20), P6 (T40), P7 (T60) and P8 (T80) released 80.26%, 72.02%, 70.48% and 80.92% of drug at the end of 24 hrs. The results are shown in Fig. 2. It was evident from the results that proniosome gels prepared with Tweens gave a higher release than Spans. This is because Tweens are more hydrophilic and have higher HLB value.

Table 2: Evaluation of Selegiline hydrochloride proniosome gel formulations.

Sl. No.	Formulation code	Colour	Vesicle size (µm) *	Rate of spontaneity* (mm^3×1000)	Entrapment efficiency* (%)	pH
1	P1	Brown	3.82±1.4	9.58±0.29	85.87±1.08	5.2
2	P2	White	4.78±1.2	8.86±0.66	88.50±1.25	5.4
3	P3	White	4.80±1.2	7.98±0.23	90.50±1.25	5.3
4	P4	Brown	2.85±1.3	9.86±0.52	82.28±0.45	5.4
5	P5	White	4.94±1.0	7.93±0.64	70.25±1.05	5.8
6	P6	Brown	5.12±1.62	9.68±0.7	73.63±4.12	6.2
7	P7	Yellow	5.04±0.95	7.28±0.22	80.24±4.72	5.5
8	P8	Yellow	5.71±1.28	6.28±0.18	81.9±4.8	6.3

**Average of three determinations±SD.*

The drug being lipophilic favors more partition in the proniosome gel in case of spans and hence less drug is released from span formulations than tweens. Proniosome gel P8 (Tween 80) released highest percentage of drug i.e., 80.92% in 24 hrs, compared to other formulations in in-vitro release studies and was considered as optimized formulation.

In vitro permeation studies

In vitro permeation of optimized gel P8 across rat abdominal skin was found to be 75.263% after 24 hrs. It was found that permeation of Selegiline hydrochloride from proniosomal gel formulation prepared with Tween was slower than span. This was probably due to the larger size of the vesicles and the less lipophilic nature of the Tweens, which makes them more difficult for the vesicles to penetrate or fuse with the skin.

In vivo studies - Anti-depressant activity

To find out the efficacy of Optimized Proniosome gel P8 in controlling depression, anti-depressant activity was evaluated using Tail Suspension Test (Fig. 3). The results are given in Table 03 and Fig. 4. The data was analysed by using Graph Pad in stat by one way analysis of variance (ANOVA) using unpaired-t test. P8 showed a significant anti-depressant activity with $P<0.05$.

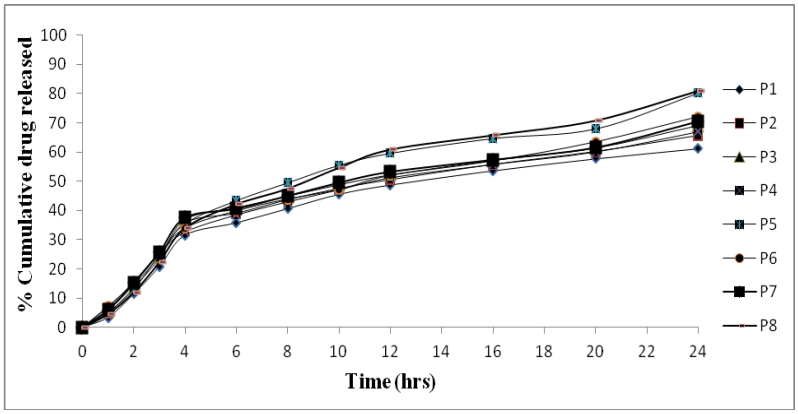


Fig. 2: Comparative in-vitro release profiles of Selegiline hydrochloride proniosome gel formulations in pH 7.4 buffer.

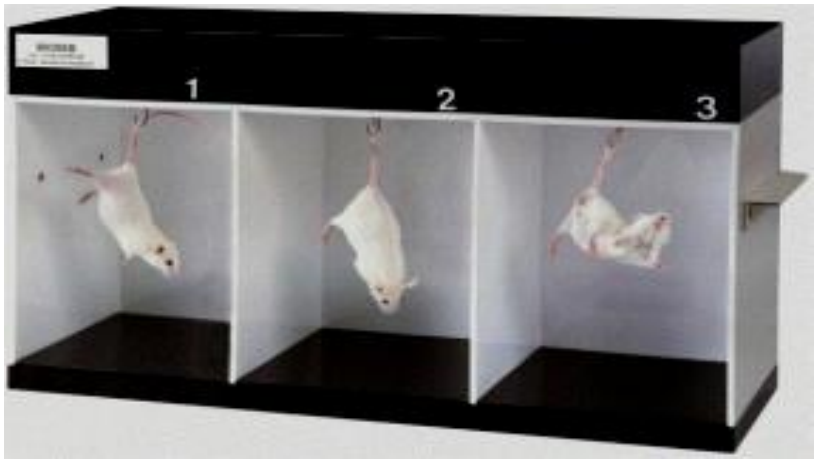


Fig. 3: Anti-depressant activity of Proniosome gel P8 by Tail Suspension Test

Table 3: Effect of Selegiline hydrochloride proniosome gel P8 on mice (Anti-depressant activity by Tail Suspension Test).

Sl.No.	Control			Test		
	Animal	Body Weight	Immobility period(Sec) [n=6]	Animal	Body Weight	Immobility period(Sec) [n=6]
1	H	23	127	A	25	160
2	B	22	145	C	24	132
3	T	24	156	D	23	140
4	H B	25	163	AC	24	110
5	BT	21	138	CD	25	120
6	HT	24	161	AD	23	98
Mean±SEM	-------		**148.3±5.796**	--------		**126.7*±9.058**

Statistical analysis followed by unpaired‘t’ test. Where * represents significant at P<0.05, ** represents highly significant at P<0.01 and *** represents very significant at P<0.001 and NS represents not significant compared to control group of animals.

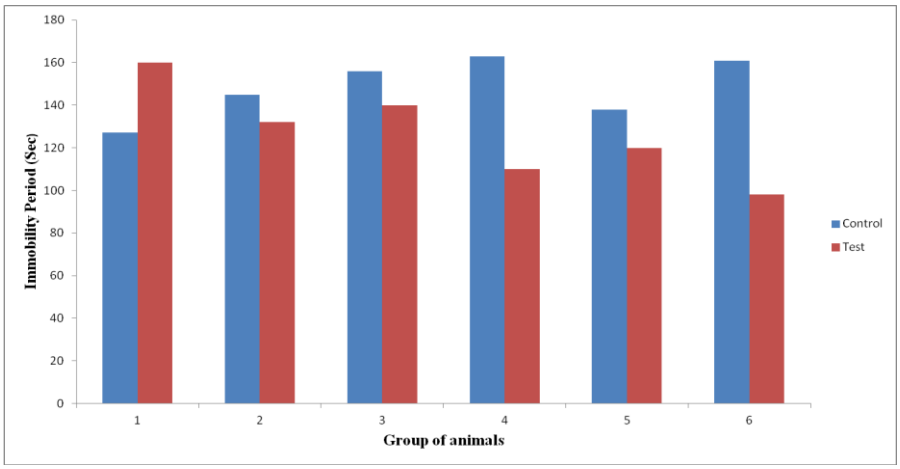


Fig. 4: Anti-depressant activity of Selegiline hydrochloride proniosome gel P8

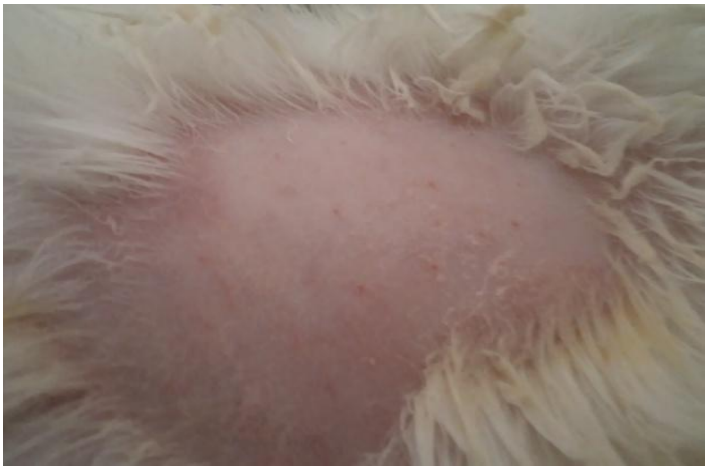
Skin irritation studies

Skin irritation study was performed using six Swiss albino rabbits to investigate whether the developed proniosome gel P8 might cause irritation or pain, after its application on the skin. The skin irritation study was performed by applying aqueous solution of formalin 0.8% as standard irritant and proniosome gel P8 as test gel. Well defined erythema was observed on skin after 24 hrs, when 0.8% formalin was applied. There was no sign of erythma or edema up to 24 hrs at the site of proniosome gel in comparison with formalin. The Primary Dermal Irritation Index (PDII) obtained for formalin 0.8% was 2 indicating it is slightly irritating because of its chemical nature. The proniosome gel P8 gave a PDII of about 0 indicating the proniosome gel belong to Non irritating class as the PDII is less than 0.5. Thus it was concluded that the proniosome gel P8 remained non irritant to rabbit skin and the results are shown in Table 4& Fig. 5.

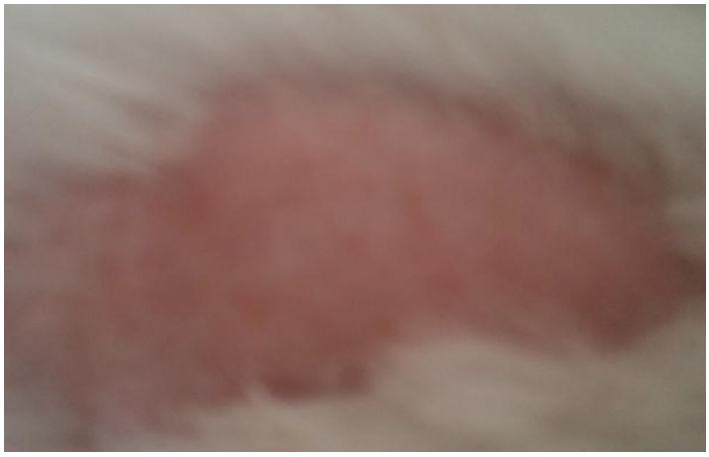
Table 4: PDII values obtained from the observation of the rabbit skin, are given below:

Subject	Erythema		Edema	
	Intial*	Final*	Intial*	Final*
Formalin 0.8%	0	**2**	0	0
Proniosome gel	0	0	0	0

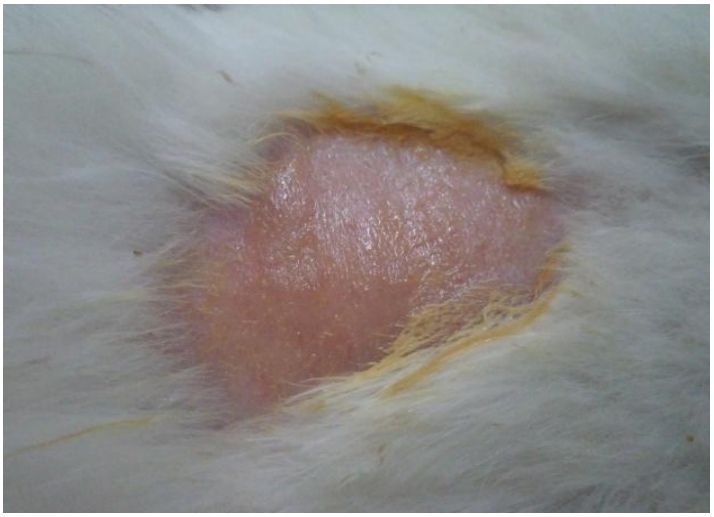
**Average of 6 readings*



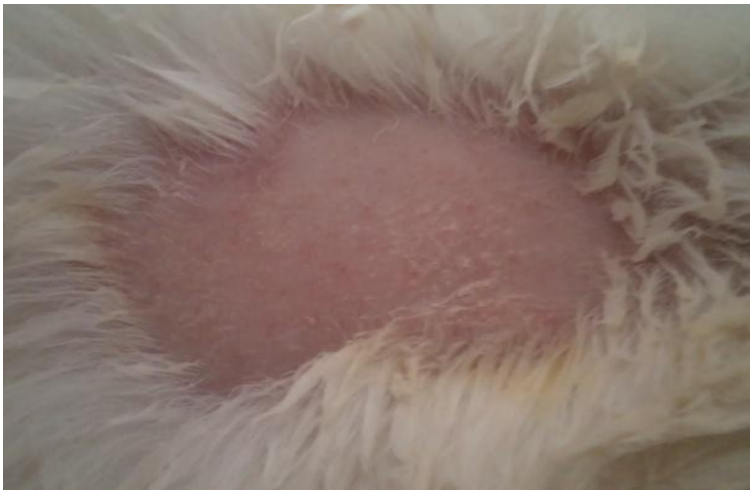
a. Before formalin



b. After formalin



c. Proniosome gel P8 applied



d. Proniosome gel P8 removed

Fig. 5: Photograph of skin irritation studies on rabbit skin.

CONCLUSION

The results of investigation demonstrated that proniosomes offers an alternative colloidal carrier approach in transdermal drug delivery. The results clearly revealed that Selegilne hydrochloride proniosomal gel prepared by coacervation phase separation method was capable of controlling and releasing drug for the extended period of time. It was concluded that Selegilne hydrochloride Pronisome gel could be of therapeutic interest in antidepressant therapy.

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