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# Effect of chemical penetration enhancers on the transdermal delivery of olanzapine in human skin in vitro

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## Abstract

This research study involves the development of an olanzapine (OLZ) formulation using various chemical penetration enhancers (CPEs) for transdermal delivery. The aim of this study was to obtain the initial data needed about the effects of various CPEs on the skin permeation of OLZ. The effects of the selected CPEs were examined by studying the permeation profiles of OLZ from formulations applied to human cadaver skin samples. A control formulation of OLZ in propylene glycol (PG) was prepared and compared against formulations containing chemical penetration enhancers. Five different CPEs (oleic acid (OA), cineole (Cin), isopropyl alcohol (IPA), Tween 80 (T80), and *N*-methyl pyrrolidone (NMP)) at 5% w/w were individually added to the formulation containing OLZ in PG. The in vitro permeation study was carried out using vertical Franz diffusion cells mounted with human cadaver skin. Samples from the receptor compartment of the cell were collected at 2 h, 4 h, 8 h, 12 h, and 24 h at room temperature. The amount ( $\mu\text{g}/\text{cm}^2$ ) of permeated drug (OLZ) was measured using a validated HPLC method, and the percentage (%) of OLZ permeated was calculated. Based on the data obtained, different CPEs were found to have a significant impact on OLZ permeability compared to the control formulation. The most effective chemical penetration enhancer was shown to be 5% w/w OA with a 3.3-fold increase in enhancement ratio (ER). The rank of order for the highest concentration of OLZ permeated from each of CPE containing formulation was as follows: OA > Cin > IPA > T80 > NMP. The most effective chemical penetration enhancer was OA but the cytotoxic study using human fibroblast cells suggests that OA may not be safe due to its cytotoxic effects.

**Keywords** Olanzapine, Schizophrenia and bipolar disorder, Transdermal, Penetration enhancer, Permeation, Solubility study, Skin deposition

## Introduction

The skin is considered one of the largest organs in the human body, which continuously serves as a self-regulating armor against the loss of water from the body (Trommer and Neubert 2006). It also prevents pollutants and other chemicals from entry into the body, regulates

body temperature, and provides a fairly robust barrier to drug permeation (Vig et al. 2017). However, the skin is an attractive route for the transdermal delivery of drugs due to its extensive surface area. Many substances, including sunscreens, antiseptic agents, and insect-repellent formulations, are designed to stay on the skin's surface (topical application), while others such as nitroglycerin and nicotine are intended to pass through the skin layers (transdermal application) in order to reach sites inside the body or underneath the skin (Supe and Takudage 2021). In other words, transdermal drug delivery can be defined as the concept by which a medication dissolves through the different skin layers and into the circulatory

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system of the body to produce a therapeutic effect (Garner and Croft 2002).

Comparing the transdermal delivery of medications to traditional pathways of administration, there are many benefits of transdermal delivery, including the absence of hepatic first-pass metabolism, decreased adverse reactions due to more stable blood level profiles, simple usage, and increased length of drug therapeutic activity (Abraham et al. 1995; Wortmann et al. 2010). However, the primary barrier component of the skin structure, which is the stratum corneum, acts as a strong defense against the permeation of a variety of substances, and this skin layer has to be altered in a non-damaging manner to make sure that drugs are transported through the skin in an adequate therapeutic amount (Kanikkannan et al. 2005).

Comprehending the physiology and chemistry of the skin is crucial for effectively utilizing the process of percutaneous drug absorption. The epidermis, hypodermis, and hypodermis (the subcutaneous layer) are the three primary histological layers that make up the skin (Benson 2005). The epidermal skin layer can be further divided into five main parts: the stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum are the layers present in the epidermis, which has a thickness of about 0.1 to 1.5 mm. The epidermis is the site of cell division, keratin and lipid production, and melanin production. The thickest layer of the epidermis is the squamous cell layer. It facilitates the movement of specific compounds into and out of the body. The stratum corneum (also known as the “horny layer”) is composed of around 10–30 thin layers of anucleated, keratinized, dead skin cells that form an organized protein, water, and lipid structure (Vardy et al. 2001; Benson 2005). The 1.5–4-mm-thick dermis is found directly below the epidermis. It has lymph and blood vessels, sweat glands, oil-producing sebaceous glands, hair follicles, collagen, and elastin. However, it is worth mentioning that drugs cannot effectively penetrate the stratum corneum, the topmost layer, which acts as a barrier within the skin (Dubey et al. 2012; Baishakhi et al. 2016). Therefore, the physicochemical characteristics of the drug have a significant impact on how much percutaneous drug absorption occurs when a topical or transdermal medication is applied. Only a small subset of medications can be administered via the transdermal pathway due to the dependence on physical and chemical characteristics of their molecules (Dubey et al. 2012; Haq et al. 2020). In transdermal drug delivery systems (TDDS), the effective penetration of drug molecule is crucial. Basically, there are several ways for drugs to penetrate the skin. Three different routes allow micro- and macromolecules to permeate the skin: (a) The lipid

matrix, which is present in the spaces between the cells of keratinocytes, serves as the intercellular route and is mostly preferred by lipophilic molecules; (b) the intracellular (transcellular) route, which passes via the corneocytes; and (c) the trans-appendageal route passes through sweat and sebaceous glands and hair follicles (Cevc 1997; Desai et al. 2010). Following topical medicine usage, molecules interact with the skin's surface where they come into contact with sebum, cellular debris, microbes, and additional substances that barely impact penetration (Moser et al. 2001). Drugs that take the intracellular route move through the stratum corneum's cells explicitly, whereas those that take the intercellular pathway penetrate slowly around the cells (Malan et al. 2002; Desai et al. 2010).

The intracellular pathway is challenging to move because it must first enter each cell's lipophilic membrane, then pass through the hydrophilic core of the cell, and finally exit the lipophilic membrane. Due to this fact, as per their polarity, both hydrophilic and lipophilic molecules prefer to pass through this route (Bolzinger et al. 2012). The intracellular pathway also needs some hydrophilic properties to pass through the corneocyte's interior. However, the intercellular pathway has an additional penetrative polar pathway. The use of absorption boosters, or substances that react with skin components to increase medicinal flow, has long been a method of expanding the variety of medicines which are successfully administered through the dermal pathway (Dragicevic et al. 2015).

The usage of penetration enhancers could significantly increase the number of actives suitable for transdermal administration (Mathur et al. 2010). According to the lipid-protein partitioning (LPP) concept, permeation enhancers employ one of three main mechanisms to exert their effects: (i) disrupting the lipid matrix of the stratum corneum, (ii) interacting with intracellular proteins (Roy et al. 2017), or (iii) enhancing the ability of the medication or solvent to penetrate into the stratum corneum. The LPP concept helps identify substances that modify the components of the stratum corneum and the structural pathway for lipid diffusion. Permeation enhancers affect the desmosomes that maintain cohesion between corneocytes, leading to fissuring of the intercellular lipids and splitting of SC squames (Williams and Barry 2012). Permeation enhancer acts by enhancing the solubility of the drug in the formulation (Dragicevic et al. 2015). Kolliphor CS 20 was proven to increase penetration of econazole nitrate through the nail (Puri et al. 2022). The penetration enhancers were able to increase the solubility of the various drug molecules. As drug concentration increases, it leads to a high concentration gradient across the skin, thus increasing the permeation. Various

formulations such as emulsion, solid dispersion, nanoe-mulsion, emulsomes, and ethosomes have been formulated to enhance solubility and bioavailability (Patel et al. 2016, Bahr et al. 2019, Parikh and Sawant 2019, Kapadia et al. 2021, Patel and Patel 2021, Ajarapu et al. 2022, Shah et al. 2022, Modi et al. 2023, Patel et al. 2023).

As absorption enhancers, a wide range of chemically structured compounds have been studied. Fatty acids and alcohols, pyrrolidones, amides, surfactants, and urea and its derivatives are some of these substances. The variances in each class of enhancer's framework and physicochemical characteristics are the key aspects that determine the extent of penetration enhancement (Williams and Barry 2012).

Olanzapine (OLZ) is an antipsychotic medication that is primarily used to treat symptoms of schizophrenia and bipolar disorder. It is available in an oral formulation. Oral administration of OLZ shows first-pass metabolism of the drug that reduced the availability of OLZ in systemic circulation by over 40% (Jawahar et al. 2018). The transdermal drug delivery of OLZ as an alternative route increases the bioavailability of the OLZ.

In the present study, we aimed to increase the low skin permeation potential of olanzapine by using various chemical penetration enhancers. This research has used five CPEs with various physicochemical characteristics to examine the efficacy and processes of olanzapine absorption across human cadaver skin. These CPEs include *N*-methyl-pyrrolidone (NMP), Tween 80 (T80), cineole (Cin), isopropyl alcohol (IPA), and oleic acid (OA).

## Materials used in study

### Chemicals

The drug substance OLZ investigated in the study was obtained from Thermo Fisher Scientific, MA, USA. Gattefosse Corporation, Paramus, NJ, USA, provided Transcutol<sup>®</sup> P (TRC) as a gift for research and development purposes. Cineole (Cin), HPLC grade acetonitrile and water, propylene glycol (PG), Tween 80 (T80), *N*-methyl-2-pyrrolidone (NMP), isopropyl alcohol (IPA), polyethylene glycol 400 (PEG 400), limonene, ethanol, and orthophosphoric acid were obtained from Sigma-Aldrich, Saint Louis, MO, USA. The phosphate-buffered saline (PBS) solution, which has a pH of 7.4, was created by dissolving one PBS tablet in 100 mL of water. PBS tablets were bought from MP Biomedicals in Solon, OH, USA. Dermatomed human cadaver skin from the posterior torso region of a 59-year-old male was purchased from New York Firefighter Skin Bank, New York, NY, USA. Note: the cadaver tissue sample was obtained from an accredited US Tissue Bank, and this is listed as exempt from the Institutional Review Board. Human dermal fibroblast (HDFa) cells were procured from the American

Type Culture Collection (ATCC), VA, USA. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific, MA, USA.

### Cell culture

A human dermal fibroblast (HDFa) cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin solution and incubated at 37 °C in an incubator with a humidified 5% carbon dioxide environment as per the ATCC guidelines. After reaching 80–90% confluency, the cells were subcultured. The HDFa cells were seeded onto 96-well plates at a density of  $8 \times 10^3$  cells per well and incubated overnight at 37 °C in 5% CO<sub>2</sub>.

## Methods

### Solubility determination

OLZ solubility was investigated in different penetration enhancers using an excess amount of OLZ in the 5-mL vehicles. An excess amount of OLZ was added to PG, Cin, OA, IPA, NMP, ethanol, PEG400, PBS, limonene, and T80 followed by vortexing for 5 min and sonication in a water bath for 60 min at room temperature. The samples were mixed for 48 h at room temperature to increase the solubility of the drug substance into the solvents (Dugar et al. 2016, Rathod et al. 2020, Virani et al. 2023a). After 48 h, the mixtures were centrifuged at 10,000 rpm to separate dissolved (supernatant) and undissolved drug (sediment). Further, the supernatants were passed through 0.2- $\mu$ m polypropylene syringe filter to remove the undissolved drug. The filtrate supernatants were diluted in the mobile phase and quantified by using the validated high-performance liquid chromatography (HPLC) method (described below).

### HPLC method

HPLC was developed to determine the concentration of OLZ in the solubility and permeation samples. The HPLC equipment used was an Agilent 1100 series coupled with UV detection (with diode array detector (DAD)) and Agilent ChemStation software (OpenLab CDS, ChemStation Edition, Rev. C.01.10, Agilent Technologies, Santa Clara, CA, USA). The method was validated with respect to linearity, precision, accuracy, and repeatability for the drug molecule. For OLZ, a standard concentration range from 0.1  $\mu$ g/mL to 100  $\mu$ g/mL was utilized to generate a calibration curve with a coefficient of correlation,  $R^2 > 0.99$ . Table 1 shows HPLC details for the quantification of OLZ.

**Table 1** HPLC details for quantification of olanzapine

HPLC column	Phenomenex C18 column 150-mm length × 4.6 mm, 5.0 μm
Mobile phase	Buffer: 10-mM disodium hydrogen phosphate buffer (pH 7.4) 30:65 (buffer: acetonitrile) ratio by volume
Flow rate	1 mL/min
Column temperature	40 °C
Wavelength	229 nm
Retention time	3.3 min
Injection volume	30 μL
Limit of detection (LOD)	0.05 μg/mL
Limit of quantification (LOQ)	0.15 μg/mL
% RSD for intra-day	0.7%
% RSD for inter-day	1.1%

### In vitro skin permeation study of olanzapine

The dermatomed human cadaver skin, obtained from the posterior torso of a 59-year-old male donor, was stored at  $\times 80$  °C until use. The sample was obtained from the New York Firefighter Skin Bank, NY, USA. Prior to the study, the skin was thawed in pH 7.4 phosphate-buffered saline (PBS) at room temperature for 5 min. The skin is then cut into approximately 2 cm<sup>2</sup> pieces (Matharoo et al. 2023) and hydrated in PBS pH 7.4 for 10 min before the permeation study. Each skin piece was mounted between the donor and receptor chambers of the Franz diffusion cells (Logan Instruments, Somerset, NJ, USA). The stratum corneum, the outermost layer of the skin, faced upward toward the donor chamber, while the lower part of the skin was in contact with the receptor chamber (Virani et al. 2023b). The diffusion area for permeation was 0.64 cm<sup>2</sup>. The receptor chamber was filled with 5.0 mL of PBS containing 30% v/v of PEG 400 at pH 7.4. A magnetic stirrer was used to continually stir the media in the receptor chamber at 600 rpm. The temperature was maintained at 32 °C  $\pm$  0.5 °C. The receptor chamber was allowed to equilibrate with the skin for 15 min before applying the dose. The donor phase, which contained 0.5 mL of a suspension of the compound of interest (OLZ) in PG, was applied to the stratum corneum in the donor chamber. The suspension was either alone or with the addition of 5% w/w of penetration enhancers. Samples of 300 μL were collected from the sampling arm of the Franz cells at specific time intervals, 2, 4, 8, 12, and 24 h. After each sampling, the collected samples were replenished with fresh receptor media which were

**Table 2** Formulation content

Formulation no	Formulation content
F1	1% w/w of OLZ in PG
F2	1% w/w of OLZ + 5% of Cin in PG
F3	1% w/w of OLZ + 5% of IPA in PG
F4	1% w/w of OLZ + 5% of NMP in PG
F5	1% w/w of OLZ + 5% of OA in PG
F6	1% w/w of OLZ + 5% of T80 in PG

PG propylene glycol, Cin cineole, T80 Tween 80, NMP N-methyl pyrrolidone, IPA isopropyl alcohol, OA oleic acid

kept at 32 °C. The cumulative amounts of drug were corrected for the sample concentrations removed from the cell receptors. All the samples collected at different time points were analyzed using a validated HPLC method described in the earlier section.

### Formulation preparation

The control formulation OLZ in the PG without penetration enhancer was prepared by addition of the 1% w/w of OLZ in PG. The test formulations were OLZ in PG with penetration enhancers, and these were prepared by addition of the 1% w/w of OLZ in PG with 5% w/w of OA, Cin, T80, NMP, and IPA alone (Table 2).

### Skin deposition quantification of OLZ

At the end of the permeation study, the skin samples were removed from Franz diffusion cells, cut around the diffusional area, and washed with ethanol to remove formulation residual. After this step, the skin samples were air-dried and accurately weighed to determine the initial weight. The dried skin samples were then placed into BeadBug tubes. The skin samples inside the tubes were cut into small pieces using scissors. To extract the drug from the skin samples, 1 mL of ethanol was added to each sample tube. These BeadBug tubes are designed for sample homogenization and extraction. The sample was homogenized using BeadBug™ Microtube homogenizer, D1030 (Benchmark Scientific, Sayreville, NJ, USA). Following that, the samples were sonicated to extract the drug from the skin. The sample tubes were centrifuged at 10,000 rpm for 10 min. Centrifugation assisted separation of the skin residue from the liquid extract. The liquid extract was then filtered through a 0.45-μm polypropylene filter (Virani et al. 2023a). The content of OLZ in the filtrate was analyzed using a validated HPLC method described in the earlier section.

### Cytotoxicity assays

To determine in vitro cytotoxicity of olanzapine and the different excipients, HDFa were treated with 0- (control)

to 250- $\mu$ M concentrations of olanzapine solution in media only and different formulations diluted in media. Cell viability was determined 24 h later using the Alamar Blue assay (Invitrogen, CA, USA). Each drug was tested in triplicate experiments repeated at least three times). The fluorescence intensity from the samples was measured by using a microplate reader (Tecan, Männedorf, Switzerland).

### Data analysis

Using the established HPLC method, the transdermal penetration of OLZ from various formulations over a 24-h period was recorded. The cumulative amount of OLZ that penetrated through a unit area of skin as a function of time was plotted to create a permeation profile. The slope of the linear component of the graph was used to determine the flux (J) value. The average OLZ flux was calculated by performing individual experiments five times. The enhancement ratio (ER) and flux (J) for each chemical penetration enhancer were calculated using the following equations:

$$ER = \frac{\text{Flux with the enhancer (test formulation)}}{\text{Flux without enhancer (control formulation)}}$$

$$J = \frac{\text{Slope of linear part of graph}}{\text{Area of diffusion cell}}$$

The statistical analysis of the data was evaluated by using one-way ANOVA and Student's *t*-test. The results were concluded statistically significant, if the *p*-value is less than or equal to 0.05. All calculations were evaluated using Statistical 12.5 software (StatSoft Inc., Tulsa, OK, USA).

### Results and discussion

The development of a suitable and improved formulation using various chemical penetration enhancers to potentially deliver olanzapine (OLZ) drug through the skin is described in the current study. This includes a selection of appropriate solubilizing agents, various chemical penetration enhancers, and a study (*in vitro permeation test*) evaluating the impact of chemical penetration enhancers on the drug's permeability through the human cadaver skin as compared to a control formulation.

#### Solubility determination of olanzapine in selected solvents

Preliminary solubility determination of OLZ was carried out using different solvents. OLZ is a highly lipophilic drug, and it belongs to the BCS Class II (low solubility, high permeability) category which exhibits poor aqueous solubility. The maximum solubility of the OLZ was observed in the solvent PEG 400 as  $45.79 \pm 1.62$  mg/mL.

The solubility results of OLZ (mg/mL) in different solubilizing agents are presented in Table 3.

The solubility of OLZ in PBS was observed as the lowest which was  $1.28 \pm 0.04$  mg/mL. The solubility of OLZ in PBS can further be increased significantly by adding 20% PEG400 ( $5.71 \pm 0.23$  mg/mL) and 30% PEG400 ( $11.50 \pm 1.67$  mg/mL). To ensure the presence of sink conditions, PBS with the addition of 30% PEG400 v/v was used as receptor media during the permeation study. Results demonstrated that OLZ showed the highest solubility in the solvent PEG 400 ( $45.79 \pm 1.62$  mg/mL) alone compared to other vehicles. The solubility results of OLZ in PEG-400 are 2.2-fold higher than IPA ( $20.05 \pm 0.40$  mg/mL) and 1.8-fold higher than oleic acid ( $24.71 \pm 1.10$  mg/mL) and at 25 °C.

Solubility of OLZ in PG ( $2.29 \pm 0.10$  mg/mL) was considered further in this research study owing to the fact that PG is the most commonly used solvent and other vehicles (OA, Cin, IPA, T80, and NMP) can be used at different stages of this research.

#### Effect of chemical penetration enhancers

The barrier challenge offered by the skin's outmost layer—the stratum corneum (SC) during the development of an optimized transdermal formulation with an appropriate penetration rate, can be overcome by the addition of various penetration enhancers to the formulation which can reversibly modify the barrier function of SC and make passage for the drug to travel through the skin into the epidermis/dermis layer (Chen et al. 2014, Chen et al. 2016, Parhi et al. 2020, Virani et al. 2023b). CPEs are widely used in dermal formulations to increase the solubility of lipophilic actives (Ezealisiji and Okorie

**Table 3** Solubility data for olanzapine in various solvents (*n*=3, mean  $\pm$  SD)

Solvents	Solubility (mg/mL) <sup>a</sup>
Water	0.01 $\pm$ 0.002
Cin	1.48 $\pm$ 0.11
PG	2.29 $\pm$ 0.10
Ethanol	8.27 $\pm$ 0.22
T80	15.03 $\pm$ 0.90
NMP	16.6 $\pm$ 0.36
IPA	20.05 $\pm$ 0.40
OA	24.71 $\pm$ 1.10
PEG 400	45.79 $\pm$ 1.62
PBS	1.28 $\pm$ 0.04
PBS + 20% PEG400	5.71 $\pm$ 0.23
PBS + 30% PEG400	11.50 $\pm$ 1.67

PBS phosphate buffer saline, PG propylene glycol, Cin cineole, T80 Tween 80, NMP N-methyl pyrrolidone, IPA isopropyl alcohol, OA oleic acid, PEG polyethylene glycol

<sup>a</sup> The values represent the concentration of OLZ in mg/mL at 48 h

2018; Hmingthansanga et al. 2022). In this study, a total of five CPEs (oleic acid, cineole, isopropyl alcohol, Tween 80, and *N*-methyl pyrrolidone) were added individually in concentration of 5% w/w to the formulation containing drug (OLZ) in PG. The suitability of each chemical penetration enhancer and its mechanism of action and success rate are further explained in this manuscript.

To evaluate the effect of CPEs on OLZ skin permeation, various CPEs were incorporated at a fixed concentration of 5% (w/w) in a PG solution. Six formulations (one control formulation and five test formulations, each containing 5% w/w of different CPEs) were developed to study skin permeation effect offered by CPEs. The permeation profile of all the formulations was studied in vitro by IVPT. Skin permeation across human cadaver skin for six formulations in five replicates, for 24 h (receptor sample interval 0, 2, 4, 8, 12, 24 h), was evaluated, and the results are summarized in Table 4.

The table shows all CPEs used in the study have significantly increased the permeation rate (transdermal flux) of OLZ. It is clear from this table that out of five CPEs used in the current study, two of the CPEs, i.e., cineole and OA, have doubled (2.0-fold) and tripled (3.3-fold) ER compared to the control formulation, respectively. No significant difference between the ERs of NMP, T80 (1.7-fold), and IPA (1.8-fold) was observed.

#### Oleic acid

Oleic acid (OA) is a naturally occurring fatty acid found in various fats and oils. OA is a mono-saturated fatty acid and is a multifunctional pharmaceutical excipient which offers the properties of being an effective chemical penetration enhancer, antioxidant, and anti-inflammatory when used in topical formulations (Atef et al. 2022). OA as a chemical penetration enhancer has shown a significant increase in SC permeation of doxycycline micro-particles containing OA as compared to the control

formulation. The study indicated OA acts by disordering densely packaged intracellular lipid domains of SC and thus allowing the drug to penetrate the SC into the dermal layers (Aliyah et al. 2021). A research study of zaltoprofen transdermal delivery has investigated the use of OA as a penetration enhancer confirming OA's dermal safety and acceptability in topical administration for not causing any skin irritation (Baek et al. 2013). A recent study of delivering adapalene topically also supports OA as an effective chemical penetration enhancer (Salimi et al. 2021). The optimized formula of ketoprofen gel containing 5% OA as a penetration enhancer exhibited improved anti-inflammatory activity compared to that available commercially (Sharma et al. 2020). OA disturbs the skin barrier function by disrupting SC lipid organization which is equivalent to increasing SC lipid temperature to 50 °C. Commercially available NSAID topical formulations contain OA as a penetration enhancer. A generic formulation of diclofenac 2% containing OA as a penetration enhancer and reference product containing diclofenac 2% containing oleyl alcohol as CPE are equivalent in in vitro penetration study determining and validating the method parameters and describing discriminatory power of the test method as well as in pivotal penetration study on industrial scale batches of generic product (Slíva 2021).

#### Cineole

One of the extensively used penetration enhancers is a monoterpene compound, i.e., cineole (Cin) which is the major component of eucalyptus oil and has been shown to increase permeation of lipophilic and hydrophilic drugs in human skin and rat skin. Cineole has shown promising results as a chemical penetration enhancer in transdermal delivery of Huperzine A through a microemulsion formulation (Shi et al. 2013; Moghimi et al. 2016). Apart from permeation enhancement property, cineole has shown a

**Table 4** Effect of penetration enhancers on permeation parameters of OLZ

Formulation	Permeated amount of OLZ ( $\mu\text{g}/\text{cm}^2$ ) Mean $\pm$ SD* Q <sub>24</sub> hours	Transdermal flux ( $\mu\text{g}/\text{cm}^2/\text{h}$ ) $\pm$ SD	Enhancement ratio (ER) <sup>^</sup>
Control PG	40.60 $\pm$ 5.00	1.71 $\pm$ 0.20	1.0
Formulation with 5% NMP	67.60 $\pm$ 6.80	2.83 $\pm$ 0.32 <sup>a</sup>	1.7
Formulation with 5% T80	68.80 $\pm$ 8.60	2.87 $\pm$ 0.25 <sup>a</sup>	1.7
Formulation with 5% IPA	74.40 $\pm$ 7.10	3.13 $\pm$ 0.25 <sup>a</sup>	1.8
Formulation with 5% Cin	81.20 $\pm$ 10.70	3.40 $\pm$ 0.41 <sup>a</sup>	2.0
Formulation with 5% OA	133.10 $\pm$ 19.10	5.60 $\pm$ 0.75 <sup>a</sup>	3.3

<sup>a</sup> The flux of OLZ increased significantly ( $p < 0.05$ ) compared to the control

\*  $n = 5$  replicates

<sup>^</sup> Enhancement ratio is calculated based on flux value

successful effect of inhibition of microorganisms such as bacteria, fungi, and parasites. This antimicrobial property of Cin is attributed to its capacity to interact with the biofilm of microorganism and extracting the essential elements of the cell and causing the ultimate cell death (Li et al. 2022). In a recent study, cineole in concentration of 5% was reported to increase the skin penetration enhancement ratio of dimethyl fumarate (DMF) to 5.3-fold (Ameen and Michniak-Kohn 2017).

#### **Isopropyl alcohol**

Isopropyl alcohol is a secondary short-chain alcohol also known as isopropanol and is widely used as a skin penetration enhancer in research as well as commercial dermal or transdermal products. It is used as a penetration enhancer in a commercial topical gel of diclofenac (trade name: Voltarol, Emugel) commercial transdermal patch of testosterone (trade name: Axiron and Tostran) along with other CPEs (Lane 2013). IPA has exhibited enhanced skin penetration and total skin absorption for the drug benzoic acid (Zhang et al. 2017). IPA was also used as a hydrophilic solvent and a penetration enhancer for the topical delivery of albumin. IPA caused an enhancement of albumin permeation (Shokri et al. 2014). IPA was used as a penetration enhancer for the aloe vera Emulgel (Mahuli and Anandamoy 2020).

#### **Tween 80**

Tween 80 is commercially used as a nonionic hydrophilic surfactant in the topical and transdermal formulations. Tween 80 is reported to extract intercellular lipids in SC and thus promote diffusion of the drugs and subsequent absorption of drugs. Tween 80 helps to achieve enhanced drug permeation through the skin by increasing the fluidity and solubility of the lipid contents of SC and by binding with keratin filaments that results in disruption within corneocyte (Kumar et al. 2015).

The synergistic effect of OA and Tween 80 for transdermal delivery of disulfiram further confirms a promising direction for the use of Tween 80 in topical formulations (Saitoh et al. 2023). In another study, Tween 80 has acted as an effective penetration enhancer for the transdermal delivery of diclofenac sodium through polymeric patches (Abdullah et al. 2023). T80 used in concentration of 1% w/w has shown increased ER and penetration for lorazepam as compared to the control sample containing water PG (50:50 v/v) (Nokhodchi et al. 2003).

#### **N-Methyl pyrrolidone (NMP)**

NMP belongs to the pyrrolidones group of penetration enhancers and is a widely studied enhancer amount in this group (Williams and Barry 2012). NMP is one of the most studied CPE which was first introduced in semisolid

preparations in the early 1980s. The increased drug penetration through the skin in the presence of NMP is attributed to NMP's ability to interact with keratins and lipids of the SC which then solubilize the lipid bilayer (Cilurzo et al. 2014).

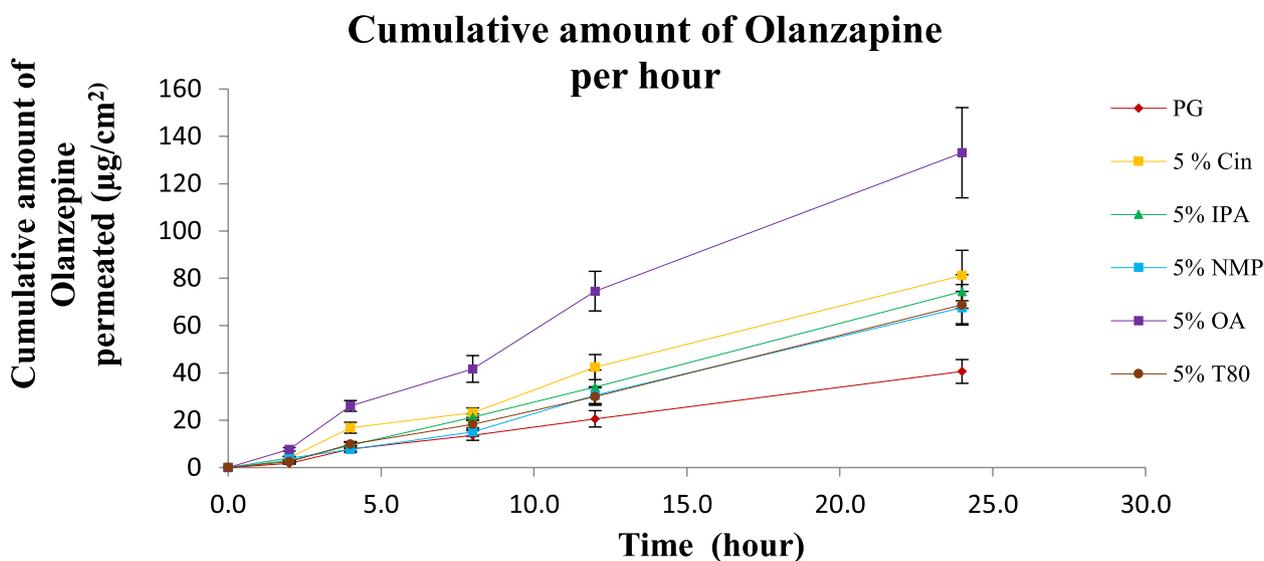
A study assessing percutaneous permeation of tetrahydropalmatine (THP) Emulgels with various CPEs including NMP showed the highest cumulative permeation amount of THP in the presence of NMP used as a chemical penetration enhancer (Li et al. 2011, Javadzadeh et al. 2015), while recently another study showed a limited success of NMP as a penetration enhancer for captopril when formulated in a matrix type transdermal patch (Ganesh Sharma et al. 2012). NMP has multiplied the transdermal flux of ibuprofen 16-folds and flux of flurbiprofen by 3-folds from films (Saini et al. 2014, Roy et al. 2017).

#### **Skin permeation studies**

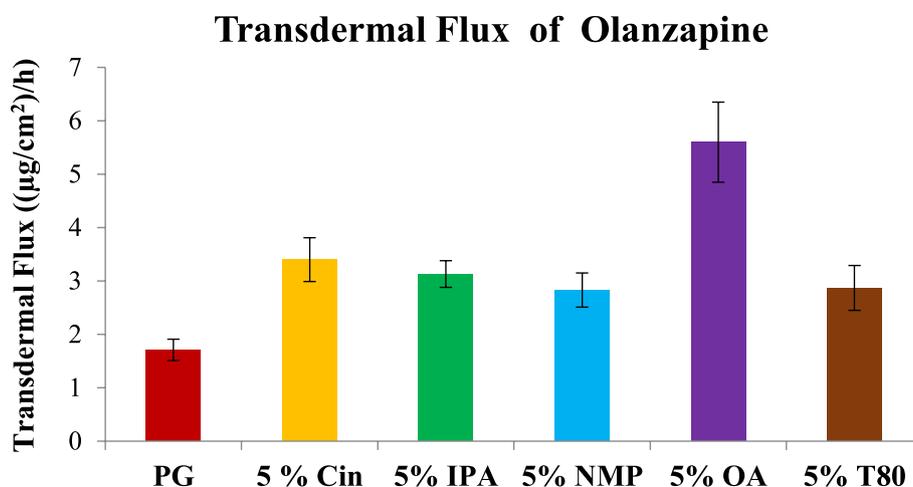
The effectiveness of the chemical penetration enhancers was determined by comparing permeation profile of OLZ with or without CPE. The cumulative amount of the permeated drug (OLZ) is plotted against time. The results of these permeation studies from all formulations are depicted in Figs. 1 and 2.

Longer time effect of each penetration enhancer can be seen from the drug permeation profile (Fig. 1). At the end of the 24-h study, the control formulation showed the lowest amount of OLZ permeated through the skin. The permeated amount of OLZ from the formulations containing 5% CPEs was significantly ( $p < 0.05$ ) higher than the control formulation and exhibited the effectiveness of the penetration enhancers. The addition of 5% OA into the formulation has resulted in a significant increase ( $p < 0.05$ ) in the amount of permeated drug ( $133.1 \pm 19.1 \mu\text{g}/\text{cm}^2$ ) compared to using the control formulation with PG ( $40.60 \pm 5.00 \mu\text{g}/\text{cm}^2$ ) after 24 h. As shown in Fig. 1, the permeated amount of OLZ from all formulations containing CPEs ranked as oleic acid > cineole > IPA > Tween 80 > NMP.

Our solubility study result indicated that OLZ has the lowest solubility in cineole as compared to NPM, T80, and IPA. However, cineole shows higher permeation than these studied permeation enhancers. In addition to solubilization, high permeation could be due to cineole possessing weaker cohesive forces, and hence, less energy is required for its molecules to H-bond with the skin ceramide polar heads which can increase the permeation (Jain et al. 2002). Our results of the skin deposition study (Fig. 3) also show that formulation with cineole has a higher amount of OLZ present in the skin layer as compared to NPM, T80, and IPA. Additionally, Ameen et al. demonstrated a comparable outcome



**Fig. 1** Mean cumulative amount of OLZ permeated through human cadaver skin for 24 h in the presence of 5% CPEs (CPEs=OA, Cin, IPA, T80, and NMP; control formulation=OLZ in PG). Each point represents the mean  $\pm$  SD of five replicates



**Fig. 2** Transdermal flux of olanzapine (drug) across the human cadaver skin using a series of penetration enhancers. Each point represents the mean  $\pm$  SD of five replicates

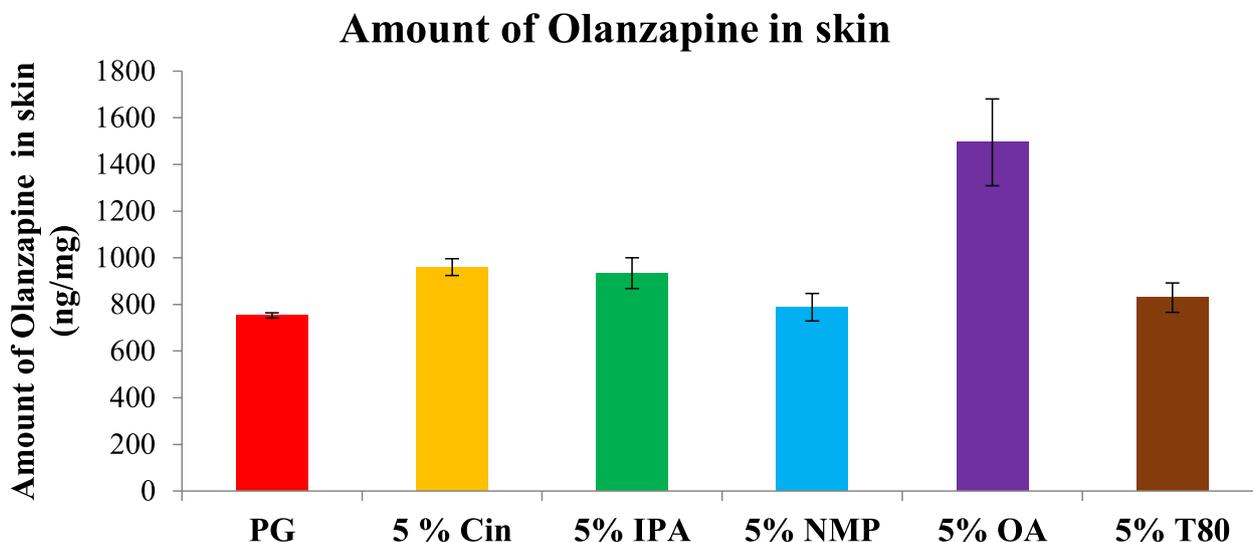
in the skin permeation of dimethyl fumarate (DMF) by cineole (Ameen and Michniak-Kohn 2017). Cineole at a concentration of 5% V/V exhibits a DMF solubility of  $13.9 \pm 0.81$  mg/mL, comparable to  $13.8 \pm 0.14$  mg/mL at 5% V/V for Transcutol P. However, the flux rates are notably higher for cineole with a value of  $108.5 \pm 17.5$   $\mu\text{g}/\text{cm}^2/\text{h}$  and  $42.1 \pm 4.81$   $\mu\text{g}/\text{cm}^2/\text{h}$  with Transcutol P.

OA was the most effective penetration enhancer exhibiting the highest transdermal flux of OLZ, i.e.,  $5.6 \pm 0.75$   $\mu\text{g}/\text{cm}^2/\text{h}$ . OA significantly increased ( $p < 0.05$ ) the flux of OLZ 3.3-fold, from  $1.71 \pm 0.2$  to  $5.6 \pm 0.75$   $\mu\text{g}/\text{cm}^2/\text{h}$ . The OLZ fluxes with cineole and

IPA were  $3.40 \pm 0.41$   $\mu\text{g}/\text{cm}^2/\text{h}$  and  $3.13 \pm 0.25$   $\mu\text{g}/\text{cm}^2/\text{h}$ , respectively. The difference between the flux of NMP ( $2.83 \pm 0.32$   $\mu\text{g}/\text{cm}^2/\text{h}$ ) and T80 ( $2.87 \pm 0.25$   $\mu\text{g}/\text{cm}^2/\text{h}$ ) was not significant ( $p > 0.05$ ).

#### Skin deposition quantification of OLZ

In Fig. 3, the amount of OLZ (measured in nanogram) detected after 24 h of permeation study through human cadaver skin (measured in mg) is presented. From the results, it is evident that the addition of 5% of OA as penetration enhancers significantly ( $p < 0.05$ )



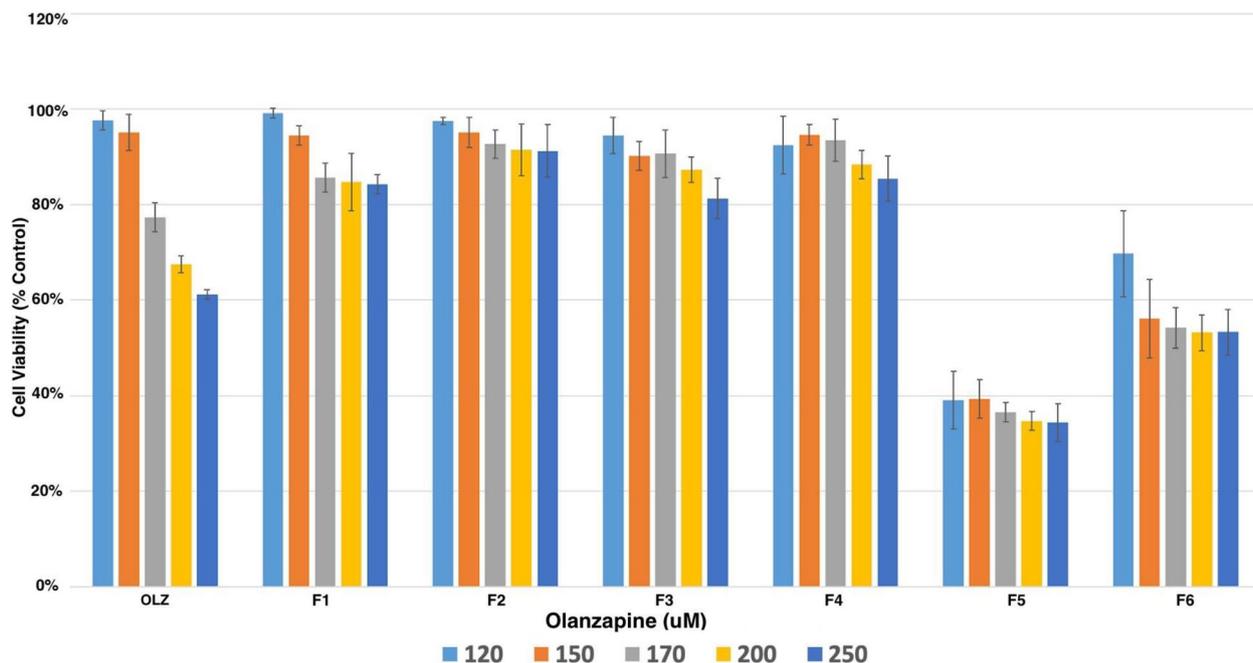
**Fig. 3** Amount of olanzapine (ng) deposited per weight of human cadaver skin (mg) post 24 h of IVPT. Each point represents the mean  $\pm$  SD of five replicates

increases the amount of OLZ in the skin as compared to control formulation.

**Cytotoxicity assays for the tested formulations**

The formulation comprising F1, F2, F3, and F4 demonstrated significant potential in a delivery system for

transdermal administration of olanzapine, along with suitable excipients. This formulation (Fig. 4) shows enhanced cell viability compared to olanzapine solution, particularly at concentrations of 170, 200, and 250  $\mu$ M. F1 employed propylene glycol as the main solvent across all formulations, which, when combined with olanzapine,



**Fig. 4** Viability and proliferation of human dermal fibroblasts (HDFa), as measured by Alamar Blue assay, were observed with varying concentrations (0–250  $\mu$ M) of olanzapine solution or in different solutions and surfactants (F1, F2, F3, F4, F5, and F6) after 24-h incubation (37  $^{\circ}$ C, 5% CO<sub>2</sub>) in DMEM medium supplemented with 10% FBS

proved to be a promising and safe option for transdermal delivery.

F2 incorporates cineole, also known as eucalyptol, which is a natural organic compound found in essential oils of eucalyptus trees and other plants (Ince et al. 2018). Extensive research has explored its diverse biological activities, including anti-inflammatory and antioxidant properties. It is suggested that cineole protects the skin from damage and contributes to increased cell viability (Ho et al. 2020). However, further studies are required to fully comprehend the potential applications of cineole in skincare formulations, specifically regarding its effects on human dermal fibroblast cells.

F3 employs isopropyl alcohol (IPA), an antiseptic agent, which was found to improve cell viability compared to olanzapine alone. Furthermore, F4, the combination of *N*-methyl pyrrolidone with propylene glycol, exhibited a favorable combination as a carrier for olanzapine, ensuring safety in transdermal delivery (Jouyban et al. 2010). Conversely, the combination of propylene glycol with oleic acid (F5) significantly decreased cell viability. This may be attributed to oleic acid's potential to induce oxidative damage in fibroblasts, as observed in a study by Romana-Souza et al. (Romana-Souza et al. 2020). Additionally, F6, the combination of propylene glycol with Tween 80, also led to reduced cell viability as reported by Hoda et al. that polysorbate 80 has minimal cytotoxic effects (Hoda et al. 2016). In summary, F1, F2, F3, and F4 all attenuated the killing effects observed with olanzapine solution. This observation suggests the potential use of these formulations in drug delivery systems for olanzapine.

Overall, the permeation of OLZ was significantly increased in the presence of the various permeation enhancers oleic acid, cineole, isopropyl alcohol, Tween 80, and *N*-methyl pyrrolidone. Furthermore, the use of these permeation enhancers in microemulsion formulation can create a synergistic effect that leads to an increment in drug permeation (Virani et al. 2023a, b).

## Conclusions

This research study was designed with the aim to collect the initial data on the effect of various chemical penetration enhancers (CPEs) on the transdermal permeability of the antipsychotic drug olanzapine (OLZ). The results of *in vitro* human cadaver skin permeation studies from all the formulations with or without CPEs were compared. According to the results, when compared with the formulation without the penetration enhancer (control formulation), the drug permeated was increased in the presence of all the other five CPEs used at a concentration of 5% w/w. The results of the present study revealed that different CPEs produced a significant effect on the penetration

of the OLZ drug through the skin. The highest permeated amount of OLZ was observed in the presence of oleic acid. However, after conducting a cytotoxicity study on the formulations, it was determined that while oleic acid showed enhanced permeation effects, it also exhibited detrimental effects on the skin. Consequently, it may not be a suitable choice as a permeation enhancer. In conclusion, transdermal delivery of olanzapine may be a promising approach to the oral route with an appropriately optimized formulation.

## Abbreviations

OLZ	Olanzapine
CPEs	Chemical penetration enhancers
PG	Propylene glycol
OA	Oleic acid, cineole
IPA	Isopropyl alcohol
Cin	Cineole
T80	Tween 80
NPM	<i>N</i> -Methyl pyrrolidone
TDDS	Transdermal drug delivery systems
LPP	Lipid-protein partitioning
PBS	Phosphate-buffered saline
HDFa	Human dermal fibroblast
ATCC	American Type Culture Collection
FBS	Fetal bovine serum
DAD	Diode array detector
SC	Stratum corneum
DMF	Dimethyl fumarate
THP	Tetrahydropalmatine

## Authors' contributions

Conceptualization, A.V.; methodology, A.V.; software, A.V.; validation, A.V. and B.M.-K.; formal analysis, A.V.; investigation, A.V.; resources, B.M.-K.; data curation, A.V.; writing—original draft preparation, A.V.; writing—review and editing, A.V., N.D., N.A., H.M. and B.M.-K.; cytotoxicity study, H.N.; visualization, A.V.; supervision, B.M.-K.; project administration, A.V. and B.M.-K.; funding acquisition, B.M.-K. All authors have read and agreed to the published version of the manuscript.

## Declarations

### Availability of data and materials

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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### Ethics approval and consent to participate

Not applicable.

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