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Research Article

Bioavailability of Bilastine Oral Self-nanoemulsion: Comparative Study with Commercial Formula in Rats

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Abstract

Background: Bilastine (BL) is a novel non-sedating second-generation antihistamine, and its bioavailability is about 60%. ***Objective***: To compare the bioavailability of prepared oral self-nanoemulsions of BL (BL-SNE) with that of pure BL and marketed tablets. ***Methods***: Four groups of Wistar rats were used in this study, each with six rats weighing between 200 and 250 g. They were treated orally using a gavage tube. The groups were fed either with conventional tablets ("Alerbix®") after being ground and dispersed with deionized water (DIW), treated with BL-SNE or fed with pure BL powder suspension. The fourth group did not receive any medication. The concentration of BL in the rat's plasma was measured using HPLC. We used Trandolapril as an internal standard. ***Results***: The bioavailability results for the prepared formula, tablet, and pure BL were 24289.91 ng/ml, 0.75 h, 12.81, 97844.7 ng.h/ml, and 98732.9 ng/ml, respectively, for the BL-SNE formula, and 15840.37, 1.0, 13.014, 66140.4, and 67088.3 for the tablets. Meanwhile, the BL suspension demonstrates 10830.12, 1.0 h, 12.96, 59397.12 ng/ml, and 60534.64 ng/ml, respectively. ***Conclusions***: The relative bioavailability of BL-SNE was 1.47 and 1.6 times higher than that of marketed tablets and pure BL, respectively. This indicates an improvement in BL's bioavailability.

Keywords: Bilastine, Bioavailability, Pharmacokinetics, Self-nanoemulsion.

تعزيز التوافر الحيوي لمستحلب البيلاستين النانوي الذاتي عن طريق الفم

الخلاصة

الخلفية: البيلاستين هو دواء حديث من الجيل الثاني لمضادات الهستامين و هو لا يتحلل بشكل كبير و لا يتفاعل مع سايتوكروم بي 450. التوافر الحيوي له حوالي 60%. **الهدف:** مقارنة التوافر الحيوي لمستحلب البيلاستين النانوي الذاتي المحضر للاعطاء عن طريق الفم بالعقار النقي والاقراص المسوَّقة. **الطريقة:** تم استخدام أربع مجموعات من جرذان ويستار في هذا البحث، كل مجموعة تضم ستة جرذان تزن بين 200-250 غم وقد عولجوا فموياً بواسطة أنبوبة خاصة . وقد أُطعمت هذه المجموعات إما بالأقراص التقليدية (اليربكس) بعد أن تم طحنها و خلطها مع الماء الخالي من الايونات أو بصيغة المستحلب النانوي الذاتي المحضر أو بمعلق االدواء النقي ولم تتلقى المجموعة الرابعة أي دواء. تم قياس تركيز الدواء في مصل الجرذان باستعمال جهاز أش بي ال سي واستُخدم دواء الترانداولابيل كمعيار داخلي لبناء منحنى معايرة نسبي لاجراء التحاليل. **النتائج:** أظهرت معلمات التوافر الحيوي للصيغ المعدة والحبوب والدواء النقي أن أعلى تركيز والزمن اللازم لاعلى تركيز وعمر النصف والمساحة تحت المنحنى من زمن صفر الى 72 ساعة و والمساحة تحت المنحنى من زمن صفر الى ما لا نهاية كانت 24289.91 نانوغرام /مل و **0.75** ساعة و0.72و 97844,7 نانوغرام * ساعة/مل و 987.32.9 نانوغرام * ساعة/مل على التوالي بالنسبة للصيغة المحضرة و 15840 نانوغرام /مل و 1 ساعة و 13.014 و 66140.4 نانوغرام * ساعة/مل و 67088.35 نانوغرام * ساعة/مل للحبوب 10830.12 نانوغرام /مل و 1 ساعة و 12.96 و 59397.12 نانوغرام /مل و 60534.34 نانوغرام /مل لمعلق البيلاستين النقي. **الاستنتاج:** كان التوافر الحيوي النسبي لصيغة مستحلب البيلاستين النانوي الذاتي أكثر بمقدار 1.47 و 1.6 مرة من الحبوب المسوقة والبيلاستين النقي على التوالي مع وجود فرق كبير بينهما ($p < 0.05$) يشير الى تحسن في التوافر الحيوي للبيلاستين.

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INTRODUCTION

Bilastine (BL) is a second-generation antihistamine. The dose for adults is 20 mg daily. It is an inverse agonist of the H1 receptor. Mast cell degranulation releases histamine, which significantly contributes to the allergic reaction. The release of histamine from mast cell degranulation significantly contributes to the allergic reaction [1]. BL binds to various H1 receptor sites and has a high specific affinity for the H1 receptor. H1 antihistamines inhibit histamine activity on sensory neurons and small blood vessels when they bind to H1 receptors, thereby reducing allergic inflammation [2]. BL is structurally distinct from other antihistamines. It is 2-[4- [2-[4-[1-(2- ethoxyethyl) benzimidazole-2-yl] piperidine-1-yl] ethyl] phenyl]-2-methylpropane acid. The BL chemical formula is C28H37N3O3 [3]. It, along with loratadine, desloratadine, and fexofenadine, are all antihistamines in the piperidine class. Compared to cetirizine and fexofenadine, the affinity of BL for the H1 receptor is 3 and 6 times higher, respectively [4]. BL is a white crystalline powder with a melting point of 203 °C and a molecular mass of 463.61 g/mol. Very slightly soluble in water. It belongs to BCS Class II. Log P is 2.41, and pKa is 4.4 [5,6]. BL fully complies with Lipinski's guidelines [7]. Like lipid formulations, isotropic self-emulsifying drug delivery systems (SNEDDS) (Figure 1) consist of appropriate oils, surfactants, and occasionally co-surfactants that, when exposed to water and lightly stirred, spontaneously emulsify to form an oil-in-water emulsion.

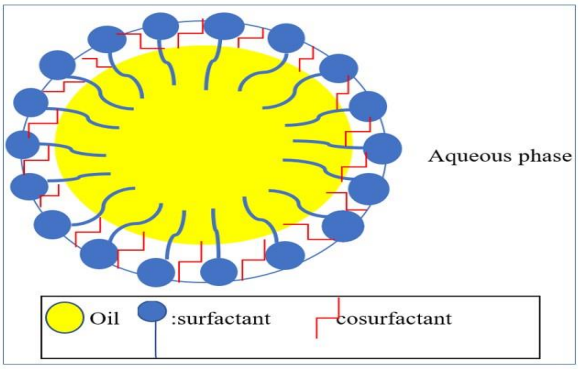

Figure 1: Isotropic structure of typical SNEDDS

Self-emulsification happens when the energy needed to increase the surface area of the dispersion is higher than what is needed for an entropy-altered dispersion to work well [8]. A self-nanoemulsifying drug delivery system has several benefits compared to traditional emulsions and other lipid carriers. These include lower energy requirements for their preparation, enhanced stability during storage, and ease of manufacturing on a large scale [9,10]. It has been shown that self-emulsifying systems make it much easier for lipophilic drugs, especially those in the II and IV classes of the BCS, to dissolve and be used by the body. This is because these systems have more isotropic properties and lower free energy on the surface area [9]. This study aims to compare the bioavailability parameters of BL after oral administration of prepared SNEDDS versus oral administration of tablets or dispersion of pure BL with deionized water.

METHODS

Materials

The following materials were used for the preparation of SNEDDS formulas: bilastine (Hyper-Chem Ltd Co., China), oleic acid oil (pharmaceutical grade) (Hunan ER-KANG Pharmaceutical Co., Ltd., China), soluplus (BASF Co., Germany), transcutol® HP (Energy Chemical Co., China) and tween 60 (Xi'an Sonwu Biotech Co., Ltd., China).

Preparation of BL-SNE

According to data obtained from preliminary work in our lab to select the best SNE vehicle, a bilastine SNEDDS formula was developed using oleic acid as an oil, tween 60 as a surfactant, transcutol as co-surfactant and soluplus as a precipitation inhibitor. We prepared the formula by mixing accurate quantities of oil, surfactant, and co-surfactant at 40 °C on a magnetic stirrer for 3 minutes, as shown in Table 1.

Table 1: Composition of Bilastine Self-nanoemulsion

Ingredients	Amounts (g)
Bilastine	0.4
Oleic acid oil	2
Tween 60	4
Transcutol	4
Soluplus	0.55

Next, we accurately weighed BL and added it to the mixture, stirring it with a magnetic stirrer at room temperature for 10 minutes at 500 rpm. We then increased the speed and duration to 1500 rpm for 20 minutes. Next, we used a prob sonicator with 30% amplitude to sonicate the formula for 5 minutes, alternating between 2 seconds of on and 2 seconds of off [11].

Bilastine Bioavailability Study

The Search Ethics Committee approved this study and conducted it in compliance with the latest WMA Declaration of Helsinki: Ethical Principles for Medical Research [12] and the US National Academy of Science's guidelines for using laboratory animals [13]. An open-label, single-dose, randomized parallel design was used for the study. Four groups of male Wistar rats weighing between 200 and 250 g were used, with six animals in each group. The rats were kept in a room with a controlled temperature (25°C) and humidity (55±5%) for a 12-hour cycle of darkness and light, deprived of food for 24 hours prior to the experiment, but allowed free access to water. They were treated orally by a gavage tube as follows: The first group was fed conventional tablets, "Alerbix®," after being ground and dispersed with deionized water (DIW). The second

group was treated with BL-SNE. The third group was fed with a BL powder suspension and the fourth group received DIW alone. The dose administered to each rat was calculated according to the following equation [14]:

HED (mg/kg) = animal dose (mg/kg) x animal K_m/human K_m

Where the HED represents the human equivalent dose. K_m is a factor with a value based on the average body surface area (BSA) calculations of humans and rats. Then the result was multiplied by 10, which is a safety factor. After oral administration, blood samples were withdrawn from each rat at time intervals of 15 min, 30 min, 45 min, 1 h, 1.25 h, 1.5 h, 1.75 h, 2 h, and 2.5 h. We drew blood samples from each rat at intervals of 3 h, 5 h, 7 h, 24 h, 48 h, and 72 h and placed them in heparinized tubes. The plasma was separated from the blood samples immediately by centrifuging them at 4500 rpm for 10 minutes. We collected and stored the plasma samples at -20 °C for drug analysis.

Assessment of plasma BL by HPLC

The chromatographic separation was performed using a Phenomenex C8 column (25 cm x 4.5 mm). The mobile phase used for analysis was sodium phosphate in 10 mM buffer (pH adjusted to 3.5 by orthophosphoric acid), methanol, and acetonitrile (60:30:10) in isocratic elusion mode while maintaining the column temperature at 30 °C. The flow rate was maintained at 1 ml / min, the injection volume was 20 µL, and the run time was 5 minutes [15]. Trandolapril was used as an internal standard [16]. The BL plasma concentration quantification was assessed using a UV-visible spectrophotometer. The lambda max used was 248 nm using methanol as a blank. Two calibration curves were instructed using BL and Trandolapril, with concentrations ranging from 5 to 20 ppm (Figures 2 and 3).

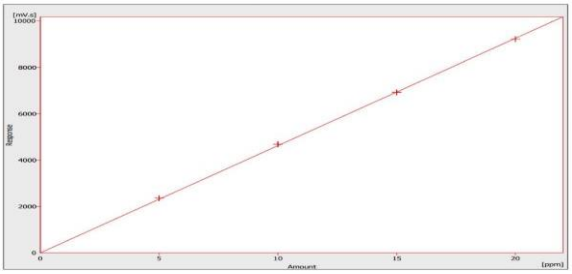

Figure 2: Calibration curve of bilastine by HPLC

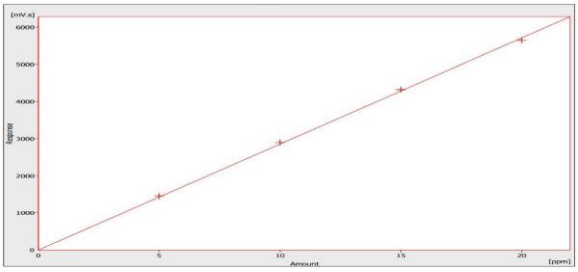

Figure 3: Calibration curve of internal standard (Trandolapril) by HPLC

We use the relative calibration curve as a reference to assess the relationship between the relative AUC and the drug's blood levels (Figure 4). C_{max}, T_{max}, $T_{1/2}$, AUC_{0-72}, and $AUC_{0-\infty}$ were analyzed statistically using a student *t*-test.

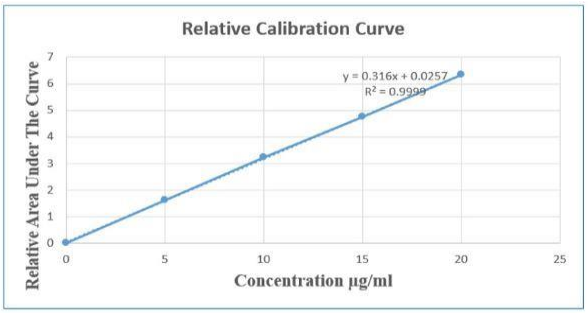

Figure 4: Relative calibration curve

Statistical analysis

The average of the triplicate samples and the standard deviation (SD) were used to represent the results. Additionally, the data were examined using paired *t*-tests at a level of 95% confidence interval ($p<0.05$) to identify significant differences. Values with $p<0.05$ are considered significantly different [17].

RESULTS

The relative calibration curve was constructed by dividing each value in the calibration curve of the standard BL solution by the values of the calibration curve of the internal standard solution. Then a new calibration curve was constructed based on the results. Figures 2, 3, and 4 illustrate the calibration curves of the bilastine solution, the internal standard solution, and the relative calibration curve, respectively. Figure 5 presented the HPLC graph of the drug-free rats' control samples, aimed at identifying any potential overlap between the BL and the control plasma.

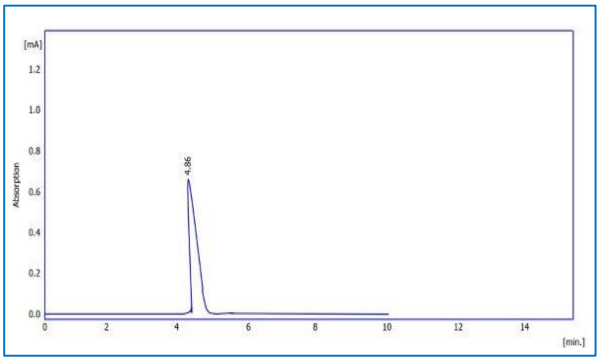

Figure 5: HPLC chromatograph of control plasma

On the other hand, figure 6 illustrates the HPLC of BL, control, and internal standards. It was clear that there was no overlapping among them since the retention time for BL was 3.58 min, which was completely different from the control (4.89 min) and the internal standard (5.31 min).

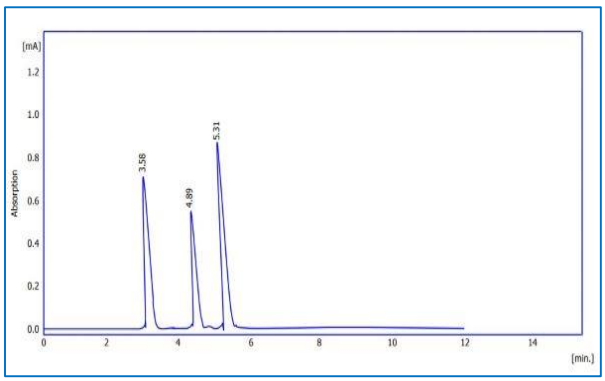

Figure 6: HPLC chromatograph of bilastine on the left, control in the middle and internal standard on the right.

In terms of BL pharmacokinetics, we calculated the relative bioavailability of oral BL-SNE compared to the pure BL powder after reconstitution with water. Figure 7 shows the drug concentration in the plasma over time after BL-SNE and BL suspension were taken by mouth. The pharmacokinetic parameters were computed using PK-Solver® (Summa Consulting Group, USA). Table 2 lists the parameters for oral BL-SNE, tablets, and BL suspensions. The significance of each value was assessed [18].

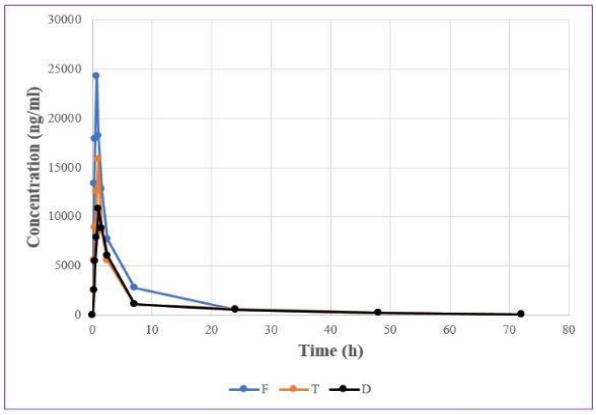

Figure 7: Mean plasma concentration of F: selected formula, T: marketed tablet and D: pure drug suspension.

The parameters mentioned in Table 2 showed that the C_{max} was 24289.91±14.32 ng/ml for the selected formula, 15840.37±15.41 ng/ml for the Alerbix® tablet and 10830.12±13.24 ng/ml for the drug suspension.

Table 2: The Pharmacokinetic Parameters of Bilastine in SNE Formula

Parameter	SNEDDS Formula	Alerbix® tablet	Drug Suspension
C_{max} (ng/ml)	24289.91±14.32[a]	15840.37±15.41[b]	10830.12±13.24[c]
T_{max} (h)	0.75±0.05[a]	1±0.4[a]	1±0.2[a,b]
$T_{1/2}$	12.81±0.72[a]	13.014±0.42[a]	13.26±0.83[a]
AUC_{0-72}	97844.7±20.43[a]	66140.4±17.32[b]	59397.12±13.41[c]
$AUC_{0-\infty}$	98732.9±18.6[a]	67088.35±14.9[b]	60534.64±16.7[c]

Values were expressed as mean±SD. Values with non-identical superscripts (a,b,c) are considered significantly different among groups (p<0.05) using unpaired *t*-test.

Statistical analysis using the t-test showed that the SNE formula was significantly better at absorption (p<0.05) than the commercial tablet and the plane BL suspension. While the T_{max} was 0.75±0.05 h, 1±0.4 h, and 1±0.2 h for the SNE formula, Alerbix® suspension, and BL suspension, respectively, with a significant difference (p<0.05) in favor of the SNE formula, AUC_{0-72}, on the other hand, was 97844.7±20.43, 66140.4±17.32, and 59397.12±13.41 for the SNE formula, 67088.35±14.9 for the marketed tablet, and 60534.64 ±16.7 for the plane BL suspension. There was a significant difference (p<0.05) between these values.

DISCUSSION

The higher C_{max} and lower T_{max} of the BL-SNE formula relative to the marketed tablets and pure BL suspension could be attributed to the high solubility of BL in the nanoemulsion. BL-SNE was 1.47 times more bioavailable than Alerbix® and 1.6 times more bioavailable than pure BL oral suspension. There was a significant difference (p<0.05) between these groups, which shows that BL was more bioavailable. Portal veins, lymphatic channels, or a combination of both could mediate the absorption of BL into the systemic circulation. Three ways exist for the lipid-based dosage form to enhance drug absorption: a) the enterocyte can absorb, release, and produce metabolites; b) the intestinal lymphatic system can transport drugs instead of the portal vein, thereby reducing first-pass drug metabolism; c) proteins and cellular junctions can aid in transport [19]. The lymphatic pathway is the preferred route for lipophilic drugs [20]. So, according to the previous mechanisms of the lipid-based dosage forms, the enhancement of the BL bioavailability may be attributed to the nanoscale size that increases the surface area of close contact between the BL-SNE formula and the epithelial membranes of the enterocytes and thus enhances oral absorption and bioavailability. Also, it is well known that when lipid-based formulas come into contact with GIT fluids, micelles are formed that help lipophilic drugs be absorbed by the body by releasing the gastric lipase enzyme [21]. In addition to the enhancement of chylomicron secretion from enterocytes by the presence of BL-SNE, which transports the drug directly through the lymphatic vessels [22], in this study, the improvement in bioavailability might be related to the presence of non-ionic tween 60 in the formula of SNEDDS. Kanwal et al. prepared the anticancer curcumin as SNEDDS and attributed the improvement in anticancer activity and bioavailability to the presence of a non-ionic surfactant tween 80, which may enhance the cell membrane fluidity and consequently the membrane permeability [23]. Rathore et al. also showed

that thymoquinone, which is anti-inflammatory, antioxidant, and hepatoprptective, was four times more bioavailable when it was made as SNE instead of the pure drug [8]. Zafar et al. noticed that the therapeutic activity and oral absorption of piperine, which is a bioactive molecule from Piper nigrum, were significantly higher (p<0.05) by preparing it as a self-nanoemulsion, and the in-vivo bioavailability in rats was 4.92 times higher than the drug dispersion [24].

Conclusion

In this study, BL-SNE, consisting of oleic acid, tween 60, Transcutol, and Soluplus as oil, surfactant, co-surfactant, and precipitation inhibitor, enhances drug diffusion and absorption across the intestinal cell membrane via various absorption routes, which appears to be a promising approach to improving the bioavailability of poorly soluble drugs.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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