Objective: The aim of the work was to study the absolute and relative bioavailability (using rabbits) of ondansetron HCl (ONH) from our newly prepared intranasal mucoadhesive nanoemulsion in situ gel (NIG) in comparison to intranasal mucoadhesive in situ gel (IG) prepared by the conventional method and intravenous injection.

Methods: Six male rabbits weighing 2.5-3 kg were used in this study, where the dose of ondansetron HCl (ONH) was calculated based on the body surface area (BSA) which is equivalent to 140μl (containing 10 mg/ml) of NIG and IG and 700μl of intravenous Zofran® injection (containing 2 mg/ml) were given to the rabbits, separated to 140μl for each week washout period. Serial blood samples were withdrawn and analyzed for the determination of the drug using HPLC (Knauere: 150 × &.6 mm; 5 μm particle size; 25 cm length) supported by guard column C18-4 mm diameter.

Results: The pharmacokinetics parameters for NIG: Cmax, Tmax, AUC0-t, AUC0-∞ were found to be greater than conventional in situ gel (IG). In vivo pharmacokinetic studies in rabbits showed a significant increase in Cmax and AUC0-∞ (P<0.001) with shorter Tmax using NIG compared to IG containing the same ONH excipients, while the absolute bioavailability for NIG and IG (was 80.541 and 51.068 respectively).

Conclusion: The present studies ratify the bioavailability enhancement potential of NE used to prepare NIG for the drug and significantly high absolute bioavailability to be used as a successful alternative route to the IV injection and improve patient compliance.

Keywords: Bioavailability study, In situ gel, Nanoemulsion, NIG, Ondansetron HCl

INTRODUCTION

Ondansetron HCl (ONH) is an antiemetic drug used as the first-line drug for the management of nausea and vomiting associated with cancer chemotherapy, chronic medical illness, gastroenteritis and post-operative states [1]. Oral ondansetron HCl (ONH) exhibits low bioavailability (i.e., 60 %) due to extensive hepatic oxidative metabolism (95%) to form hydroxylated metabolites, which is mediated through multiple cytochrome P450 forms. The myriad drug delivery systems of ondansetron HCl (ONH) developed so far have yielded limited fruiton in oral bioavailability enhancement [2].

Drugs administered through nasal route absorb rapidly and reach therapeutically effective plasma levels quickly due to the rich vasculature and highly permeable membranes in the nasal cavity [3]. Moreover, the nasal route offers an additional advantage over the oral route, particularly for those drugs that have poor oral bioavailability due to pH instability and enzyme degradation in the gastrointestinal tract (GIT). Further, drugs that undergo gut wall metabolism, P-glycoprotein efflux, and high hepatic first-pass metabolism are also benefited from nasal route of administration [4]. For a few drugs, plasma drug profiles from intranasal delivery are akin to that of intravenous drug profile of the same drug [5].

Recently, nanoemulsion (NE) is being seen as a carrier for intranasal delivery of drugs as it is more stable than other vesicular systems like liposomes, ethosomes, niosomes, etc. They are translucent mixtures of oil, surfactant, cosurfactant, and water, in which either the oil globules are dispersed in water (o/w) or water globule are dispersed in oils (w/o). These systems are single, optically isotropic and thermodynamically stable with a droplet size typically in the range of 10-100 nm [6].

Due to low bioavailability of ondansetron HCl (ONH) and its inability to reach high concentration in plasma after oral administration, it was exocicted to develop a novel dosage form for ondansetron HCl (ONH) that could address these problems through delivery of the drug into intranasal route to improve its bioavailability (by avoidance of first-pass metabolism).

However, owing to the anatomical and physiological conditions of the nasal cavity, intranasally administered drugs have low residence time at the site of administration. Rapid mucociliary clearance (MC) also limits the time available for drug absorption [4]. Therefore, in the design of intranasal formulations, one must address the problem of low residence time in the nasal cavity. Possible strategy to improve nasal residence time is to use formulations with higher viscosity, and preferably, with mucoadhesive character. Designing an intranasal dosage form with mucoadhesive character is particularly helpful because it provides intimate contact between the dosage form and nasal epithelium thereby maximizing the chances of absorption for a drug molecule. Temperature sensitive in situ gel provide a suitable dosage form; they are easy to administer and have a suitable viscosity at body temperature to counter mucociliary clearance (MC) [7].

In our previous studies, a mucoadhesive nasal in situ gel for ONH was prepared through the application of NE technology (NIG) where poloxamer 407 was used as thermo reversible polymer and chitosan and HPMC E15 were used as mucoadhesive polymers, NIG was evaluated in vitro and gave 100% release after 6 h with sharp burst effect. In addition, a nasal in situ gel (IG) containing the same contents of the nanoemulsion in situ gel(NIG) prepared by the conventional method and tested in vitro to show 60% release after 6 h [8, 9].

This study involves the in vivo estimation (for the first time) of the contribution of nanoemulsion (NE) as a carrier for ondansetron HCl (ONH) in our newly developed mucoadhesive intranasal in situ gel through studying the bioavailability of the drug relative to the intranasal in situ gel prepared by the conventional way (without carrier)as well as through comparing its bioavailability from these preparations to intravenous injection (Zofran®) of the drug.
Materials and Methods

Materials

Ondansetron HCl [Hangzhou Hyper chemicals, China], HPMC E15, poloxamer407 and chitosan [H-imedia Laboratories Pvt. Ltd., Mumbai, India], tween 20 [J. T Baker, China], acetaminophen and non-halogen absolute-HPLC grade [Biosolve B V, France], oleic acid [Al-Emad company, Iraq], Tetra-methyl-buty1 ether-HPLC grade [Raaj Guokang Biotechnology Co, Ltd. China]. For all the experiments, deionized distilled water (DDW) was used.

Methodology

Study design

The protocol for the study was approved (approval number 205/2017) by animal care committee in the Iraqi national center for drugs control and researches. Briefly, six male, white rabbits weighing 2.5-3 kg were caught in rabbit hatch during blood samples collection. The rabbits were then positioned on a table supported in a horizontal position. The nasal and intravenous dose of rabbits were calculated, an equivalent amount of ondansetron HCl (ONH) were prepared by spiking blank plasma with the working solutions (after evaporation of methanol) to get dilution (ten times) of 700 μl Zofran® injection, prepared NIG and conventionally prepared IG formulations were carried out using one-way analysis of variance (ANOVA) followed by tukey-multiple comparison test. Statistical calculation was carried out using Graph Pad Prism 7.0 software.

RESULTS AND DISCUSSION

Bioavailability study

The concentration of ondansetron HCl (ONH) in rabbit’s blood was determined using a reproducible, sensitive and rapid HPLC method. Fig. 1 showed peak chromatograms for ONH in standard conditions and in a working plasma sample, the chromatographic conditions and extraction procedure yielded a clear chromatogram for analysis.

The retention time of ondansetron HCl (ONH) was 3 ±0.5 min for ondansetron (absolute bioavailability) to estimate its use as alternative dosage form than IV injection to be safe medicated and improve patient compliance.

Materials and Methods

The calibration curve was constructed by plotting area of the calibration curve of ondansetron HCl (ONH) in plasma. The lower limit of detection of the drug in plasma by HPLC was 0.5 ng/ml. The concentration of ondansetron HCl (ONH) in rabbit’s blood was determined using a reproducible, sensitive and rapid HPLC method. Fig. 1 showed peak chromatograms for ONH in standard conditions and in a working plasma sample, the chromatographic conditions and extraction procedure yielded a clear chromatogram for analysis.

The retention time of ondansetron HCl (ONH) was 3 ±0.5 min for ondansetron (absolute bioavailability) to estimate its use as alternative dosage form than IV injection to be safe medicated and improve patient compliance.
Fig. 1: Representative HPLC chromatograms of standard solution (STD) and working plasma sample of ondansetron HCl (ONH)

![Representative HPLC chromatograms of standard solution (STD) and working plasma sample of ondansetron HCl (ONH)](image)

Fig. 2: Calibration curve of ondansetron HCl (ONH) in plasma by HPLC

![Calibration curve of ondansetron HCl (ONH) in plasma by HPLC](image)

**Fig. 3** depicts the mean plasma concentration profile as a function of time for the optimized nanoemulsion in situ gel (NIG) in comparison to prepared conventional in situ gel (IG) and in comparison to marketed IV injection (Zofran®) by using crossover design with one week washout period. The mean blood concentration versus time profile revealed that first order pharmacokinetic model without lag time and first-order elimination rate was considered to be the best fit to explain the generated data, where the drug concentration in plasma after administration of the dose and progressing of analysis, the concentration increased until reached the peak level ($C_{\text{max}}$). After the peak level of the drug was reached, the plasma level of the drug decreased indicating that the rate of elimination from plasma stream is greater than the rate of absorption (absorption process does not terminate).

Table 1 illustrated the arithmetic mean and standard deviation values of plasma drug concentration (ng/ml) of ondansetron HCl (ONH) at the selected time intervals for 6 rabbits having different weights (2.5-3 kg) and receiving single dose of ONH (1.4 mg) of the prepared nanoemulsion in situ gel (NIG), conventional prepared in situ gel (IG) and commercial Zofran® injection with one week washing period between them.

Table 2 shows the pharmacokinetic parameters for ondansetron HCl (ONH) in the prepared nanoemulsion in situ gel (NIG), conventional prepared in situ gel (IG) and marketed IV injection. From these results, it was evident that, the absorption of ondansetron HCl (ONH) from the nanoemulsion in situ gel (NIG) reached its peak plasma concentration in 2 h, the mean $T_{\text{max}}$ of marketed Zofran IV injection was 0 h (the drug directly inside the blood circulation without absorption) while, for the conventionally prepared in situ gel (IG), the $T_{\text{max}}$ was 3 h. Also, it was found that the absolute bioavailability ($F_{\text{absol}}$) for nanoemulsion in situ gel (NIG) (80.541%) was significantly higher ($P<0.001$) than that for conventional in situ gel (IG) (51.068%). These findings ensure the goal of contribution of nanoemulsion technology applied for the situ gel preparation in NIG which showed higher and rapid absorption than conventional in situ gel (IG) formula, estimated by increasing the mean maximum plasma drug concentration ($C_{\text{max}}$) and reducing the time required to reach maximum plasma drug concentration ($T_{\text{max}}$), increasing the total amount of ondansetron HCl (ONH) present in blood for longer time as indicated by the high value of $AUC_{\alpha}$ and finally, this *in vivo* study is inconsistent with the *in vitro* release of the drug from both formulas nanoemulsion in situ gel.
Nanoemulsion technology used in NIG improved the bioavailability of the drug (80.541%) through improving its release and permeability through nasal cavity [9]. In comparison to the reported work for the same drug but is prepared as intranasal surface engineered nanostructural lipid carrier showed absolute bioavailability (68.24%) which is less than that of nanoemulsion in situ gel (NIG) [19]. Also, NIG exceeds the absolute bioavailability of oral tablet and oral solution (65% and 71% respectively) [20].

The nanoemulsion in situ gel (NIG) formula remains in the nose of the experimented rabbit till the end of the study indicating good mucoadhesive property of the preparation without drainage or detachment during the experimental time. Therefore, this study showed that in situ gel nasal preparation prepared by applying nanoemulsion technology (NIG) which is liquid at room temperature and upon instillation into the nose, it will change to a gel as an acceptable alternative dosage form (to IV) with effective, safe, good bioavailability, less frequent doses (improves patient compliance) and better result than the in situ gel prepared by conventional method (IG).

### Table 1: Mean plasma concentration (ng/ml) of ondansetron HCl (ONH) at the time intervals following administration of single dose of the prepared nanoemulsion in situ gel (NIG), conventionally prepared in situ gel (IG) and the marketed Zofran® IV injection

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IV</th>
<th>NIG</th>
<th>IG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>7000±141.80</td>
<td>780.93±48.5</td>
<td>200.91±28.05</td>
</tr>
<tr>
<td>0.25</td>
<td>6850.84±71</td>
<td>1299.5±22.38</td>
<td>381.96±42</td>
</tr>
<tr>
<td>0.5</td>
<td>6482.38±49.99</td>
<td>1644.8±12.34</td>
<td>521.22±57.72</td>
</tr>
<tr>
<td>1</td>
<td>5992.7±92.14</td>
<td>2443.1±49.2</td>
<td>807.27±78</td>
</tr>
<tr>
<td>2</td>
<td>5189.48±71.17</td>
<td>3865.25±63</td>
<td>1827.4±49</td>
</tr>
<tr>
<td>3</td>
<td>4371.0±64.69</td>
<td>363±53.9</td>
<td>1985.6±49.31</td>
</tr>
<tr>
<td>5.5</td>
<td>3257.87±57.02</td>
<td>2890±82.12</td>
<td>1918.06±42.68</td>
</tr>
<tr>
<td>7.5</td>
<td>2465.86±14.83</td>
<td>2375±93</td>
<td>1409.5±57.98</td>
</tr>
<tr>
<td>10</td>
<td>1700.44±92</td>
<td>1650±129.7</td>
<td>1025.7±49.57</td>
</tr>
<tr>
<td>24</td>
<td>222.5±283</td>
<td>250±49</td>
<td>188.9±49</td>
</tr>
</tbody>
</table>

n=6 (mean±SD)

### Table 2: Pharmacokinetics parameters of a single dose of ondansetron HCl (ONH) from the optimized nanoemulsion in situ gel (NIG), optimized in situ gel (IG) formula and the commercial Zofran® IV injection

<table>
<thead>
<tr>
<th>The mean pharmacokinetic parameters</th>
<th>IV (±SD)</th>
<th>NIG (±SD)</th>
<th>IG (±SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>0 ± 0.0</td>
<td>2 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>7000±141.80</td>
<td>3865.25±67</td>
<td>2885.6±49.31</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;1-24&lt;/sub&gt; (ng/ml h)</td>
<td>50816.412±19.9</td>
<td>45165.610±38.0</td>
<td>25250.765±22.9</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;1→α&lt;/sub&gt; (%CV) (ng/ml h)</td>
<td>52428.731 (7.51%)</td>
<td>45165.610 (5.59%)</td>
<td>26774.152 (2.17%)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>K&lt;sub&gt;e&lt;/sub&gt;±SEM (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.138±0.020</td>
<td>0.092±0.041</td>
<td>0.124±0.040</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>5.022</td>
<td>7.533</td>
<td>5.589</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>%F&lt;sub&gt;absoluta&lt;/sub&gt; (IG/NIG)</td>
<td>86.147</td>
<td>51.058</td>
<td>59.28</td>
<td>-</td>
</tr>
<tr>
<td>%F&lt;sub&gt;relative&lt;/sub&gt; (IG/NIG)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n=6 (mean±SD, (1) CV% = Percent coefficient of variation. (2) SEM = Standard error mean, (3) P = Probability value

Fig. 3: Mean plasma drug concentration profile versus time obtained during the in vivo pharmacokinetic studies carried out in six rabbits receiving single dose of the prepared nanoemulsion in situ gel (NIG), conventional prepared in situ gel (IG) and marketed (Zofran®) IV injection
CONCLUSION
This work describes a fast, sensitive and robust method to quantify ondansetron HCl (ONH) in rabbit plasma using HPLC. The described method for ONH quantification in rabbit plasma was successfully applied in a bioequivalence study of the prepared nanoemulsion in situ gel (NIG), conventionally prepared in situ gel (IG) and marketed Zofran IV injection using an open, randomized, two-period crossover design with a 7 d washout period between doses. Since the prepared NIG formula showed high absolute bioavailability suggesting it as a good alternative candidate for IV injection that will improve patient compliance. The work also proved the role of nano-sized droplets in the in situ gel nasal prepared using nanoemulsion technology in improving the absorption rate and extent of the drug in comparison to conventionally prepared in situ gel preparation.

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AUTHORS CONTRIBUTIONS
All the author have contributed equally

CONFLICT OF INTERESTS
Declared none

REFERENCES