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# In Vivo Brain-Distribution Studies of Rutin Loaded Nanoemulsion for Nose to Brain Delivery

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## ABSTRACT

**Plan:** In Vivo Brain-Distribution Studies of Rutin loaded Nanoemulsion for Nose to Brain Delivery.

**Preface:** The aim of present investigation is to develop in vivo Brain-Distribution analysis for nose to brain targeting of Rutin loaded Nanoemulsion (RU-NE) via olfactory and trigeminal nerve pathways.

**Methodology:** For nose to brain drug delivery analysis in terms of DTP (Direct Nose to Brain Transport Percentage) & DTE (Drug Targeting Efficiency).

**Outcome:** These findings are in consequence with related reports by that nanoemulsions increase nose-to-brain uptake of drugs.

**Keywords:** In Vivo Brain-Distribution, Drug Targeting Efficiency, Plasma Samples, Brain Homogenates, Nasal Bioavailability.

## 1. INTRODUCTION

Rutin (RU) was anticancer agent were incorporated in nanoemulsion (Rutin (RU) loaded nanoemulsion (NE)) for nose to brain delivery via olfactory and trigeminal nerve pathway to avoid transport of RU in blood brain barrier (BBB). The developed intranasal nanoemulsion (NE) was also subjected for in vivo brain distributions studies using wistar albino rats. The results obtained from in vivo brain distributions studies were plotted as brain and plasma concentration vs time. The pharmacokinetics parameters including $T_{max}$, $C_{max}$ and AUC were estimated by Kinetica 5.0® computer program. The results of in vivo brain distributions studies showed that intranasal nanoemulsion were able to promote drug in olfactory region to allow CNS targeting and to remarkably improve the CNS concentration of the drug. This study was demonstrated rapid and larger extent of selective curcumin in nose-to-brain transport when compared with PDS in rats.

## 2. Material and Method

### 2.1. Material

Rutin (RU) supplied as a gift sample by Sunpure Extracts Pvt. Ltd (Delhi, India) was used as working standard.

### 2.2. Method

#### 2.2.1. In Vivo Brain-Distribution Studies

The *in vivo* studies were performed according to the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The animal protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, registration number 651/PO/ReBi/S/02/CPCSEA.

*In vivo* pharmacokinetic study was carried out using male Wistar albino rats. This *in vivo* brain-distribution studies can be divided in two groups each group consist of six animals. To Group First, 100 µl of the formulation (RU-NE) containing 5 mg of RU are instilled nostril with the help of micropipette attached with LDPE tubing, having 0.1 mm internal diameter the delivery site. The rats are held from the back in slanted position during intranasal (IN) administration.

For second group 100 µl of the plain drug suspension (PDS) containing 5 mg of RU are instilled into the intravenously (IV) with the help of tuberculin syringe (1 mL), at the delivery site. The rats was euthanatized by using $CO_2$ chamber (carcass disposal: Deep Burying under Soil) - disposal post-experimentation. The rats are sacrificed at different time intervals and the animals were decapitated immediately after blood collection (by retro-orbital plexus puncture) and skull was cut open and the brain was carefully excised. Each brain tissue is quickly rinsed with saline. The brain tissue samples were homogenized with 1 volume of saline in a tissue homogenizer (Figure 1 Figure 2 and Figure 3). Blood samples were anticoagulated with heparin and centrifuged at 5000 rpm for 10 min to obtain plasma. At each time point, 6 rats are taken for measurements. All plasma samples and brain homogenates were stored for up to 48 h in a deep freezer (−70˚C) until HPLC analysis.

2.2.2. Sample Processing

To 100 µl of brain homogenate or 100 µl of plasma sample, 100 µl IS Hydrochlorothiazide (20 µg/ml) and add extraction solvent 2 mL of acetonitrile was spiked and vortex mixture for 20 min. This sample was ultracentrifuge at 10,000 rpm for 10 min. The supernatant layer was collected and 20 µl was injected in HPLC system and the whole procedure was carried out at room temperature.

2.2.3. Chromatographic conditions

The chromatographic separation was performed at ambient temperature with reversed-phase, 150 X 4 mm base specific column packed with 5µm C18 column (Eclipsed XDB 5 µm, 4.6 mm x 150 mm, Singapore). The mobile phase was a mixture of acetonitrile: water with 0.1% formic acid (30:70 v/v) pumped at a flow-rate of 0.2 mL/min. Detection was performed at a wavelength of 242 nm.

2.2.4. Data Analysis

The non-compartmental model was considered as a best suited model for calculation of the different pharmacokinetic parameters. The $C_{max}$ and $T_{max}$ were directly computed from the concentration vs. time plot. The trapezoidal method was used to calculate the concentration-time curve (AUC0→t). The Kinetica 5® (Thermo Fisher Scientific Demo version) software was employed for study. The absolute nasal bioavailability of RU from nanoemulsion was calculated.

Absolute bioavailability (F) = $[AUC]_{oral} D_{i.v.} / [AUC]_{i.v.} D_{oral} * 100$ ------- (1)

Where, $D_{i.v.}$ = i.v. dose of drug, $D_{oral}$ = oral dose of drug, $AUC_{oral}$ = AUC of oral administered drug, $AUC_{i.v.}$ = AUC of IV administered drug.

To evaluate the brain targeting after nasal dosing, two indexes were adopted:

**1) Drug Targeting Efficiency (DTE)**

**DTE represents a time-average partitioning ratio,**

$$DTE\% = (AUC_{brain} / AUC_{blood})_{in} / (AUC_{brain} / AUC_{blood})_{iv} \times 100 \text{ ----- (2)}$$

**2) Direct Transport Percentage (DTP)**

In order to clarify nose to brain direct transport more clearly, we introduced a term of nose to brain drug,

$$\% DTP = [(B_{in} - B_x) / B_{in}] \times 100 \text{ ------- (3)}$$

**Where,** $Bx = (B_{i.v.} / P_{i.v.}) \times P_{i.n.}$

$B_x$ is the brain AUC fraction contributed by systemic circulation through the BBB following intranasal administration,

$B_{i.n.}$ is the $AUC_{0–120}$ (brain) following intranasal administration,

$B_{i.v.}$ is the $AUC_{0–120}$ (brain) following intravenous administration,

$P_{i.n.}$ is the $AUC_{0–120}$ (blood) following intranasal administration,

$P_{i.v.}$ is the $AUC_{0–120}$ (blood) following intravenous administration,

AUC is the area under the curve.

## 3. RESULTS AND DISCUSSION

*3.1. In-vivo* Brain distribution study in rats

The results of brain distribution studies showed the time profile of rutin concentration in brain and plasma higher after intranasal (IN) administration of drug loaded NE (RU) as compared to intravenous (IV) administration of plain drug solution (PDS) respectively. The profiles of RU level in brain displayed an initial absorption phase and maximum concentration achieved after about 20 min in brain after IN administration. These findings are in good agreement with that previously reported by for the intranasal administration of cocaine and support the existence of a nose to brain direct pathway. After the initial 20 min, the drug concentration in the brain was found higher for IN delivered RU (8328.67 ± 995.05 ng/mL) at $T_{max}$ 20 ± 8.66 than the IV administered PDS (462.73 ± 37.82 ng/mL) at $T_{max}$ 15 ± 0.00 (Table 1). The profiles of RU level in Plasma displayed an initial absorption phase and maximum concentration achieved after about 15 min in brain after IN administration. After the initial 15 min, the drug concentration in the plasma was found higher for IN delivered RU (5507.48 ± 541.84 ng/mL) at $T_{max}$ 15 ± 0.00 than the IV administered PDS (3957.38 ± 656.85 ng/mL) at $T_{max}$ 15 ± 0.00 (Table 2). The highest concentration was observed in the plasma after IN administered NE as compared to IV administered PDS. A statistically significant difference ($P < 0.05$) between the two formulations was found from the Student t-test analysis.

Significant enhancement of rutin delivery from RU-NE to the CNS when IN administered to rats as compared with IV administered PDS. This could be related to the rapid absorption and longer residence time of the RU-NE in the rat nasal cavity, which provides the opportunity for intranasal delivery to the brain via olfactory pathway. Thus, the results of the present investigation proved that drug could be transported directly to the CNS after intranasal delivery of RU-NE, thereby enhancing drug concentration in the brain and also enhancing the nasal bioavailability of rutin.

3.2. DTP (Direct Nose to Brain Transport Percentage) & DTE (Drug Targeting Efficiency)

For nose to brain direct transport following IN delivered nanoemulsion, we introduced a term of DTP (Direct Nose to Brain Transport Percentage) & DTE (Drug Targeting Efficiency). The DTP % represents the percentage of drug directly transported to the brain via the olfactory pathway. The RU-NE, RU showed the highest DTE % (1871.26 ± 2.56) and DTP % (95.21 ± 0.93) (Table 3) suggesting that RU-NE was maximum brain targeting efficiency mainly DTP via the olfactory region of the nasal cavity. These findings are in consequence with related reports by that nanoemulsions increase nose-to-brain uptake of drugs.

**Table 1: Pharmacokinetic parameters following intranasal (IN) administration of RU loaded NE and Intravenously (IV) administered RU-PDS (BRAIN)**

| Pharmacokinetic Parameters | Intranasal (IN) administration of RU loaded NE | Intravenous (IV) administration of RU-PDS |
|---|---|---|
| $C_{max}$ ± SD (ng/ml) | 8328.67 ± 995.05 | 462.73 ± 37.82 |
| $T_{max}$± SD (min) | 20 ± 8.66 | 15 ± 0.00 |
| $AUC_{0-120}$ (ng/ml) | 675797 ± 23173.10 | 27534.16 ± 472.58 |

**Table 2: Pharmacokinetic parameters following intranasal (IN) administration of RU loaded NE and Intravenously (IV) administered RU-PDS (PLASMA)**

| Pharmacokinetic Parameters | Intranasal (IN) administration of RU loaded NE | Intravenous (IV) administration of RU-PDS |
|---|---|---|
| $C_{max}$ ± SD (ng/ml) | 5507.48 ± 541.84 | 3957.38 ± 656.85 |
| $T_{max}$± SD (min) | 15 ± 0.00 | 15 ± 00 |
| $AUC_{0-120}$ (ng/ml) | 424861 ± 10717.33 | 209954.7 ± 47409.92 |

**Table 3: Drug targeting efficiency and Direct nose to brain transport following**

| Intranasal administration of optimized RU-NE | | |
|---|---|---|
| **Rutin Nanoemulsion** | **% DTE** | **% DTP** |
| Rutin | 1871.26 ± 2.56 | 95.21 ± 0.93 |

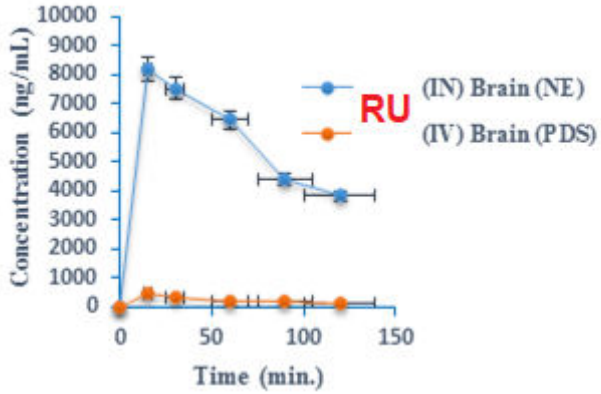


**Figure 4: Brain concentration–Time profiles of RU after IN administration of drug loaded NE and IV administration of PDS in rats respectively**

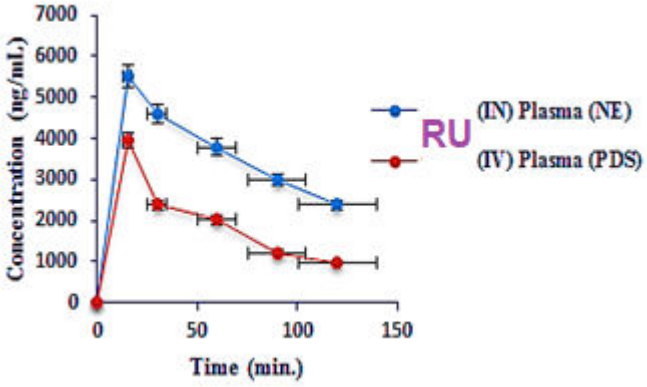


**Figure 5: Plasma concentration–Time profiles of RU after IN administration of drug loaded NE and IV administration of PDS in rats respectively**

## 4. CONCLUSION

The result of present investigation shows that RU loaded NE for intranasal administration was very promising approach of CNS targeting for the treatment of brain tumor, in particular for producing the cytotoxic effect.

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