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IN VIVO BRAIN-DISTRIBUTION STUDIES OF CURCUMIN LOADED NANOEMULSION FOR NOSE TO BRAIN DELIVERY

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ABSTRACT

Plan: *In Vivo* Brain-Distribution Studies of Curcumin 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 curcumin loaded nanoemulsion (CRM-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) and 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 and Nasal Bioavailability.

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INTRODUCTION

Curcumin (CRM) was anticancer agent were incorporated in nanoemulsion (curcumin (CRM) loaded nanoemulsion (NE)) for nose to brain delivery via olfactory and trigeminal nerve pathway to avoid transport of CRM 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.

MATERIAL AND METHODS

Material

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

Method

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 and 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, 100µl of the formulation (CRM-NE) containing 5 mg of CRM 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 100µl of the plain drug suspension (PDS) containing 5mg of CRM are instilled into the intravenously (IV) with the help of tuberculin syringe (1mL), 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 No.1, Figure No.2 and Figure No.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.

Sample Processing

To 100µl of brain homogenate or 100µl of plasma sample, 100µl IS Hydrochlorothiazide (20µg/ml) and add extraction solvent 2mL 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 20µl was injected in HPLC system and the whole procedure was carried out at room temperature.

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 5µm, 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 0.2mL/min. Detection was performed at a wavelength of 242nm.

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 CRM 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:

Drug Targeting Efficiency (DTE)

DTE represents a time-average partitioning ratio,

DTE% = (AUC $_{brain}$ /AUC $_{blood}$) *in* / (AUC$_{brain}$ /AUC $_{blood}$) *iv* × 100 ----- (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}] ×100 ------- (3)

Where, Bx = (B $_{i.v.}$ / P $_{i.v.}$) × 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,

AUCis the area under the curve.

RESULTS AND DISCUSSION

In-vivo Brain distribution study in rats

The results of brain distribution studies showed the time profile of curcumin concentration in brain and plasma higher after intranasal (IN) administration of drug loaded NE (CRM) as compared to intravenous (IV) administration of plain drug solution (PDS) respectively. The profiles of CRM 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 CRM (8328.67 ± 995.05ng/mL) at T_{max} 20 ± 8.66 than the IV administered PDS (462.73 ± 37.82ng/mL) at T_{max} 15 ± 0.00 (Table No.1, Figure No.4). The profiles of CRM 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 CRM (5507.48 ± 541.84 ng/mL) at T_{max} 15 ± 0.00 than the IV administered PDS (3957.38 ± 656.85ng/mL) at T_{max} 15 ± 0.00 (Table No.2, Figure No.5). 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 curcumin delivery from CRM-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 CRM-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 CRM-NE, thereby enhancing drug concentration in the brain and also enhancing the nasal bioavailability of curcumin.

DTP (Direct Nose to Brain Transport Percentage) and 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) and DTE (Drug Targeting Efficiency). The DTP% represents the percentage of drug directly transported to the brain via the olfactory pathway. The CRM-NE, CRM showed the highest DTE% (1871.26 ± 2.56) and DTP% (95.21 ± 0.93) (Table No.3) suggesting that CRM-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 No.1: Pharmacokinetic parameters following intranasal (IN) administration of CRM loaded NE and Intravenously (IV) administered CRM-PDS (BRAIN)

S.No	Pharmacokinetic Parameters	Intranasal (IN) administration of CRM loaded NE	Intravenous (IV) administration of CRM-PDS
1	C_{max} ± SD (ng/ml)	8328.67 ± 995.05	462.73 ± 37.82
2	T_{max}± SD (min)	20 ± 8.66	15 ± 0.00
3	AUC_{0-120} (ng/ml)	675797 ± 23173.10	27534.16 ± 472.58

Table No.2: Pharmacokinetic parameters following intranasal (IN) administration of CRM loaded NE and Intravenously (IV) administered CRM-PDS (PLASMA)

S.No	Pharmacokinetic Parameters	Intranasal (IN) administration of CRM loaded NE	Intravenous (IV) administration of CRM-PDS
1	C_{max} ± SD (ng/ml)	5507.48 ± 541.84	3957.38 ± 656.85
2	T_{max}± SD (min)	15 ± 0.00	15 ± 00
3	AUC_{0-120} (ng/ml)	424861 ± 10717.33	209954.7 ± 47409.92

Table No.3: Drug targeting efficiency and Direct nose to brain transport following Intranasal administration of optimized CRM-NE

S.No	Curcumin Nanoemulsion	% DTE	% DTP
1	Curcumin	1871.26 ± 2.56	95.21 ± 0.93

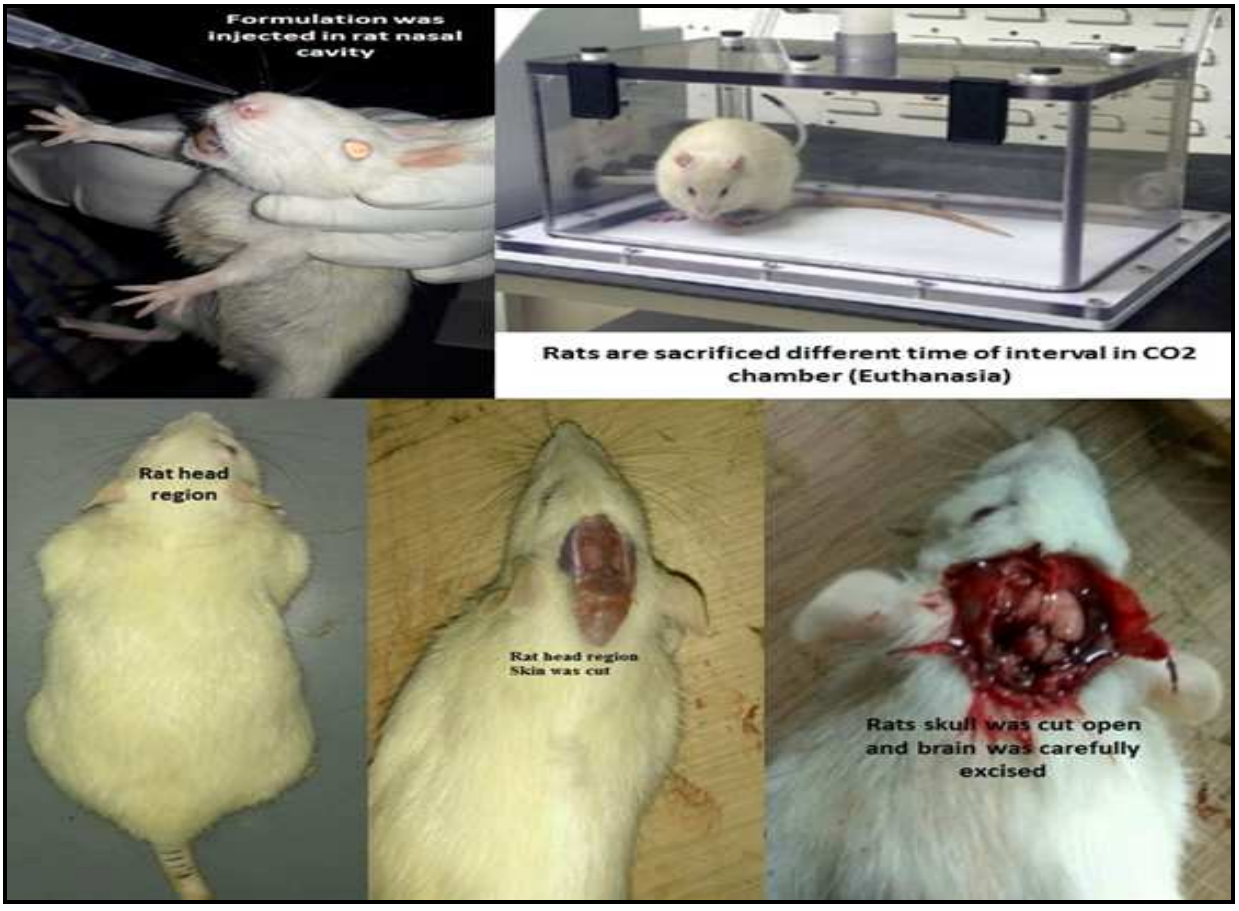

Figure No.1: Rat Intranasal administration - Formulation (CRM-NE)

Figure No.2: Rat Intravenous administration – Plain dug suspension (CRM-PDS)

Figure No.3: Rat Plasma distribution studies of CRM-NE and CRM-PDS in IN and IV route of administration

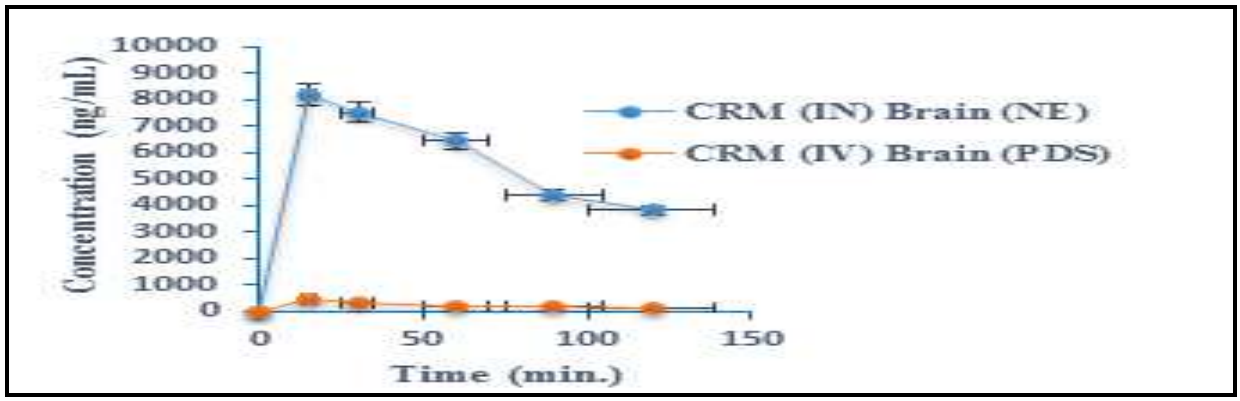

Figure No.4: Brain concentration–Time profiles of CRM after IN administration of drug loaded NE and IV administration of PDS in rats respectively

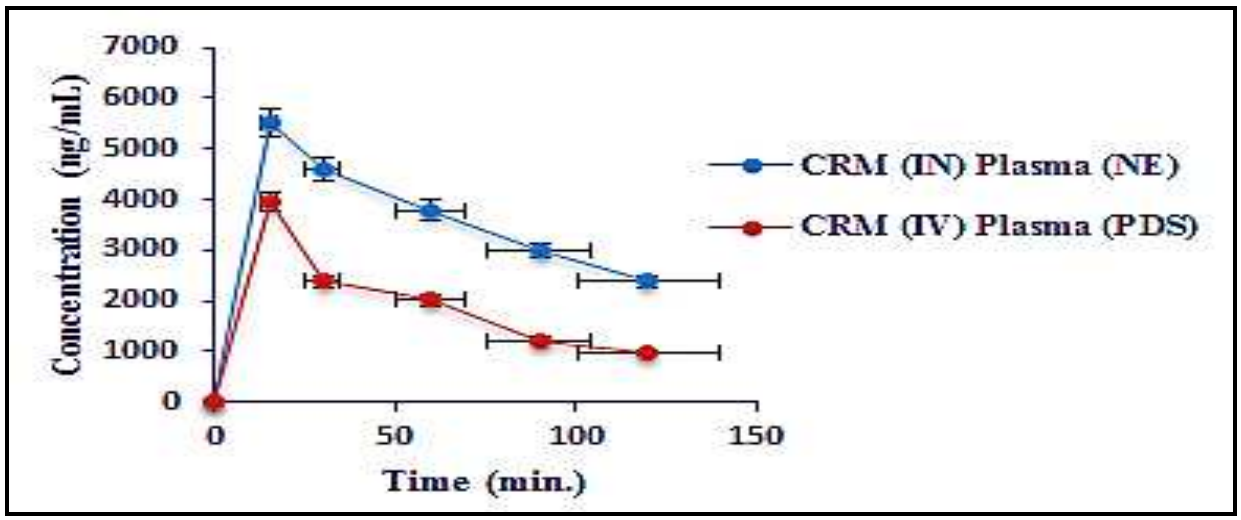

Figure No.5: Plasma concentration–Time profiles of CRM after IN administration of drug loaded NE and IV administration of PDS in rats respectively

CONCLUSION

In present operation it can be concluded that CRM loaded NE is very novel approach for nose to brain targeting via olfactory and trigeminal nerve pathways.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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