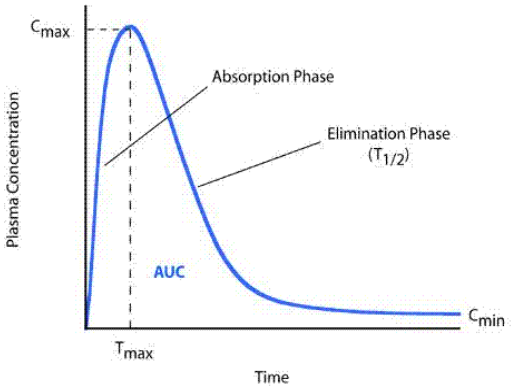


# Chapter 8 (Part- A)

# *In Vivo* Pharmacokinetic Study for Oral SMEDDS

## Management of Dyslexia and ADHD

## 8.1 Introduction

### *In Vivo* Pharmacokinetic Study for Oral SMEDDS

One can select different animal models for kinetic study but due to practical feasibility, most widely used models are rat and mice. However, rat model is usually selected over mice model due to ease of administration of formulation and organ collection. Even though number of animal studies have reduced by the *in vitro* techniques, *in vivo* studies still contribute significantly to understand toxicology, pharmacology and efficacy of drugs in development. (1) The present study was performed to understand the effectiveness of oral delivery of Modafinil SMEDDS as compared to oral delivery of Modafinil suspension and Modafinil marketed formulation. In brief, animals were divided in three groups; i) Modafinil suspension, ii) Modafinil SMEDDS, and iii) Modafinil marketed formulation. In each group, six Male Sprague-Dawley rats, each of average weight 340-360 gm were selected. Pharmacokinetic data was obtained by Kinetica 5.0 software and comparison of pharmacokinetic result (AUC, Cmax, Tmax, $t_{1/2}$, MRT) for all groups was done statistically by one way ANOVA followed by post hoc bonferroni test using graph pad prism 6.0. (2)

## 8.2 Material and Instrument

**Table 8.1 List of Materials, Glassware and Instruments used for *In Vivo* study**

| Materials, Glassware and Equipments | Manufacturer / Supplier |
|---|---|
| Centrifuge | Remi Instrument, Mumbai, India |
| pH meter | Lab India Pvt. Ltd, Mumbai |
| Bath Sonicator | Remi Equipments Pvt. Ltd, India |
| HPLC-UV detector | Shimadzu UV- 1700, Japan |
| Digital Weighing Balance | Shimadzu, Japan |
| Methanol, Acetonitrile, Acetic acid | Spectrochem, Vadodara, India |
| Beakers, volumetric pipettes, flasks, Capillary. | Borosil Glassware, India. |
| Micropipettes (10 µl,200 µl & 1ml) and Eppendorf Tubes (1.5 ml, 2 ml & 10 ml) | Tarson Pvt. Ltd., India. |

Double distilled water was prepared in the laboratory.

## 8.3 Animals

The experimental protocol for the animal study was followed as per the guidelines of institutional animal ethics committee (IAEC). The animal study protocol was approved by IAEC (IAEC Reg. No.: MSU/PHARM/IAEC/2011/15). Male Sprague-Dawley rats weighing 340-360g were selected for the study and had free access to food and water. (2) The rats was given standard rat chow and water. The rats were housed in cages placed in an animal room with a constant temperature of 22 °C and a fixed 12-hour light-dark cycle. They were fasted overnight prior to experiment but water was available ad libitum. (3) After acclimatization rats were randomly allocated to 3 groups which are as follows: (n=6 for each group). Dose of Modafinil was calculated using following formula:(4)

Dose for Animal (mg/kg) / Dose for Human (mg/kg) = Human Factor / Animal Factor. Where, weight of the rat was 350-360 gm, Human Factor was 37 and animal factor for Rat was 6.

**Table 8.2 Groups of Animal Model for Pharmacokinetic Study**

| Sr. No. | Modafinil Pharmacokinetic Study | |
|---|---|---|
| | **Experimental Group** | **Number of animals** |
| 1 | Modafinil Suspension | 6 |
| 2 | Modafinil SMEDDS (Diluted) | 6 |
| 3 | Modafinil Marketed Formulation | 6 |
| | Total No. of Animals | 18 |

## 8.4 Administration and Blood collection

The rats were divided into 3 groups and fasted overnight prior to experiments. Each oral formulation was administered via oral gavage (n=6/formulation; Dose 10.278± 0.02mg/kg). Basic information regarding the *in vivo* highlighted in table 8.3. Blood samples (0.5 ml) were collected from the retro-orbital plexus at 0.5, 1, 2, 4, 6, 8, 10 and 24 hours into the heparinized tubes post dose under light anaesthesia. (2) The plasma was collected by centrifugation at 4000 rpm, 4ºC for 10 min. The collected plasma was stored at -20°C until further analysis by using HPLC method. (2, 5, 6)

**Table 8.3 Basic Information Regarding Drug Administration**

| | |
|---|---|
| **Substances** | Modafinil SMEDDS/Marketed Formulation/Suspension |
| **Dose** | 10.278 ± 0.02 mg/kg |
| **Route of Administration** | Oral |
| **Volume** | Calculated according to body weight of animal not exceeding 1 ml. |
| **Blood Withdrawal** | |
| **Volume** | 0.5 ml |
| **Blood Withdrawal Site** | Retro-orbital plexus |

## 8.5 Analysis of Blood Samples

Supernatant (plasma) was stored at -20°C, thawed at room temperature for further analysis. In 0.5 ml of plasma equal quantity of the organic solvent (500µL acetonitrile) was added, to precipitate the protein. Again, samples were centrifuged at 4 ºC; 4000 rpm for 15 min. Clear supernatant was collected and filtered through syringe filter. The samples were analyzed into HPLC system after suitable dilution. (5, 6) Mobile phase selected for analysis contain Water: Methanol: Acetic acid (500:500:1, v/v) with 1.0 mL/min flow rate and 20 µl was injection volume. The retention time of Modafinil was 8.57 min. The λmax for the determination of Modafinil in rat plasma was 220 nm. The chromatograms were analyzed by using LC solution software and the concentration of Modafinil was calculated as we had discussed in the Chapter 4.

## 8.6 Pharmacokinetic Parameters

All the Pharmacokinetic parameters were calculated by non-compartmental analysis (extra vascular) using Kinetica software (version 5, Therma Scientific, Philadelphia, PA, USA) and recorded in tables 8.5 for SMEDDS, Suspension and marketed formulation. (7)

## 8.7 Result and Discussion

**Table 8.4 Plasma Drug Concentration**

| Time (hr) | Plasma Drug Concentration (µg/ml) | | |
|---|---|---|---|
| | Suspension | Marketed | SMEDDS |
| 0 | 0.0 | 0.0 | 0.0 |
| 0.5 | 02.858 ± 2.44 | 03.289 ± 1.56* | 09.197 ± 1.09** |
| 1 | 03.338 ± 2.53 | 07.196 ± 1.94* | 18.367 ± 1.53** |
| 2 | 04.045 ± 2.32 | 11.475 ± 2.35* | 16.387 ± 2.05** |
| 4 | 02.743 ± 0.69 | 09.625 ± 1.64* | 12.448 ± 1.99** |
| 6 | 01.830± 1.06 | 07.814 ± 1.08* | 10.084 ± 2.87** |
| 8 | 01.126 ± 1.54 | 05.381 ± 0.57* | 08.327 ± 1.53** |
| 10 | 01.039 ± 2.22 | 03.625 ± 1.19* | 06.481 ± 1.02** |
| 24 | 0.0 | 0.0 | 0.0 |

Each point represents Mean ± SD (n=6)

One way, Anova followed by post hoc Bonferroni test using graph pad prism 6.

*p<0.5, **p<0.01, ***p<0.001 compared to suspension.

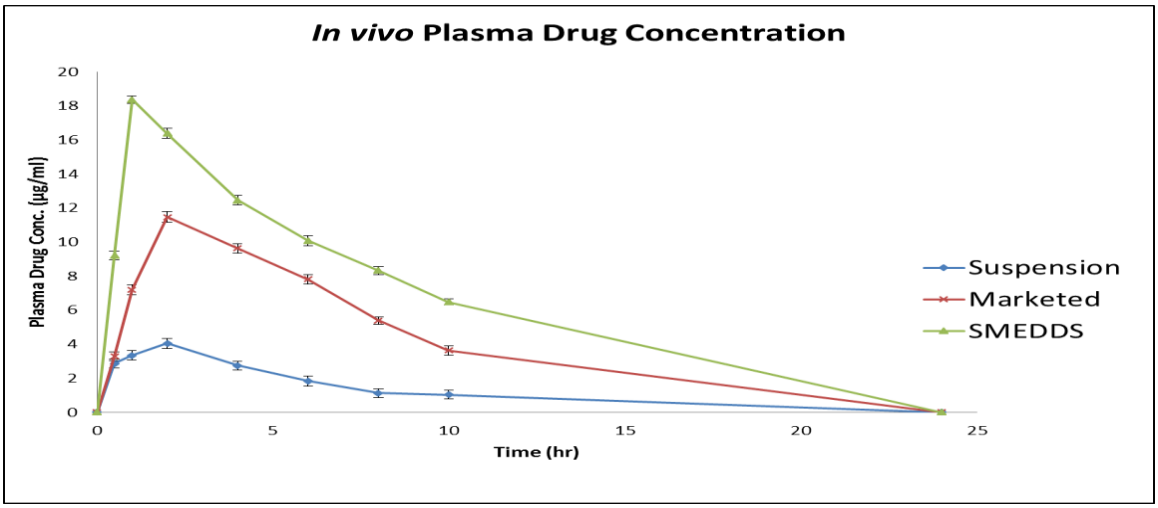


**Fig 8.1 Plasma Drug Concentration vs Time Profiles of Modafinil after Oral Administration of Suspension, Marketed Product and SMEDDS**

Result represented in fig. 8.1, shows plasma concentration vs time curve in rats after single oral dose of Modafinil loaded SMEDDS, drug suspension and Modafinil marketed formulation. At all the indicated time points, the Modafinil concentrations in rat plasma were significantly higher in rats treated with SMEDDS than those treated with marketed formulation and suspension.

**Table 8.5 Pharmacokinetic Parameters for Various Formulations of Modafinil after Orally Administered (10.278 mg/kg) into Healthy Rats**

| Pharmacokinetic Parameters | Suspension | Marketed | SMEDDS |
|---|---|---|---|
| **Cmax (µg/ml)** | 4.40 ± 01.02 | 11.48± 02.16** | 18.37± 02.04*** |
| **Tmax(hr)** | 2.00 | 2.00 | 1.00 |
| **AUC (µg/ml*hr)** | 29.51 ± 04.58 | 98.51± 08.93*** | 156.11± 09.78*** |
| **AUMC (µg/ml*hr$^2$)** | 167.56 ± 10.33 | 624.58 ± 11.85*** | 1549.53± 63.83*** |
| **$t_{1/2}$** | 3.38 ± 00.64 | 3.61 ± 00.54* | 6.06± 02.15* |
| **MRT (hr)** | 6.14 ± 02.19 | 6.78 ± 03.41 | 9.26± 03.44* |
| **Relative Bioavailability (Fr)** | ------ | 333.86 ± 17.23 | 529.05± 28.85 |

Each point represent Mean ± SD (n=6)
One way anova followed by post hoc bonferroni test using graph pad prism 6.
*p<0.5, **p<0.01, ***p<0.001 compared to suspension.

The pharmacokinetic parameters for Modafinil drug suspension, marketed tablet (converted to suspension) and SMEDDS are listed in Table 8.5. AUC is utilized to determine bioavailability of drug from dosage forms. The Cmax, AUC, MRT and Relative Bio-availability (Fr) for SMEDDS were significantly higher than that of drug suspension and marketed dosage form. Cmax and AUC for SMEDDS was about 1.6 times higher compared to the marketed formulation and 4.1 times than suspension while Tmax was reduced by half i.e. from 2hr in marketed and suspension formulation to 1hr in SMEDDS. $t_{1/2}$ of SMEDDS increased by about 1.7 times compared to marketed formulation and 1.8 times to that of suspension leading to increase in circulation time of the SMEEDS formulation. This also lead to increase in MRT of SMEEDS formulation by approximately 1.4 times to the marketed and 1.5 times to that of suspension. Increase in all the above parameters contributed to 1.6-fold surge in bioavailability of SMEDDS compared to the marketed formulation whereas 5.3 times to that of suspension. This indicated that SMEDDS achieved higher amount of drug in a short time lapse to the systemic circulation after oral administration. The higher Cmax was achieved with higher values of AUC, MRT and Fr observed in SMEDDS, suggest improvement in the rate and extent of drug concentration in the systemic circulation and finally result in increase of bioavailability.

# Chapter 8 (Part- B)

# *In Vivo* Pharmacokinetic Study for NTB Microemulsion

## Management of Dyslexia and ADHD

## 8.9 Introduction

## *In Vivo* Pharmacokinetic Study for Intranasal Vinpocetine Formulations:

The principle and objectives of the pharmacokinetic studies are to estimate the factors involved in the absorption, distribution and elimination (metabolism and excretion) and/or to compare the bioavailability of the active ingredient from two or more product formulations and/or routes of administration (bioequivalence studies). The animal species, group size, age and sex should be well defined and should be carried out in a sufficient number of healthy animals. Suitable biological fluids (blood, plasma, serum, urine, etc.) and tissues more often selected for pharmacokinetic investigation. In addition, plasma is generally considered to be the most useful biological fluid for such studies. Standard equations or equivalent calculations should be used to calculate pharmacokinetic parameters and interpretation provided of the values obtained. Various substances, including protein and peptides and some therapeutic agents have been shown to enter the brain via the olfactory pathways. (8) The present study was undertaken to find out efficacy and enhancement in bioavailability of Vinpocetine through nasal route as compared to direct transportion of drug to the brain after nasal administration.

## 8.10 Material and Instrument

**Table 8.6 List of Materials, Glassware and Instruments used for *In Vivo* Study**

| Materials, Glassware and Equipments | Manufacturer / Supplier |
|---|---|
| Acetonitrile, KH2PO4 | Spectrochem, Vadodara, India |
| Diethylamine | S.D. Fine chemical, India |
| All other glassware and equipments were same as listed in Table 8.1 | |

## 8.11 Animals

The experimental protocol for the animal study was followed as per the guidelines of institutional animal ethics committee (IAEC). The animal study was performed with IAEC approval No. (IAEC Reg. No.: MSU/PHARM/IAEC/2011/15). The Male Sprague-Dawley rats weighing 300-340g were selected and maintained as per the procedure mentioned in section 8.3. The rats was divided into four groups containing six in each.

After acclimatization rats were randomly allocated to 3 groups each of plasma and brain homogenate analysis, which are as follows:

Dose (0.514 ± 0.02 mg/kg) of Vinpocetine was calculated using following formula: (9)

Dose for Animal (mg/kg) / Dose for Human (mg/kg) = Human Factor / Animal Factor

Where, weight of the rat was 350-360 gm, Human Factor was 37 and animal factor for Rat was 6.

**Table 8.7 Groups of Animal Model for Pharmacokinetic Study**

| Sr. No. | Vinpocetine Pharmacokinetic Study (Plasma) | |
|---|---|---|
| | **Experimental Group** | **Number of animals** |
| 1 | Vinpocetine ME | 6 |
| 2 | Vinpocetine MME | 6 |
| 3 | Vinpocetine suspension | 6 |
| | Total No. of Animals | 18 |
| **Sr. No.** | **Vinpocetine Pharmacokinetic Study (Brain)** | |
| | **Experimental Group** | **Number of animals** |
| 1 | Vinpocetine ME | 18 |
| 2 | Vinpocetine MME | 18 |
| 3 | Vinpocetine suspension | 18 |
| | Total No. of Animals | 54 |

## 8.12 Administration and Blood Collection

The rats were anesthetized using chloroform inhalation from chloroform-soaked cotton. 30µL of ME/MME was administered as a dose through nostril (15 µL each), using a PE-10 tube attached to micropipette. Formulation (15 µL per nostril 0.514 mg/kg) was instilled into each nostril with the help of micropipette (1µL to10µL) attached with low-density polyethylene tubing, having 0.1mm internal diameter at the delivery site.

For oral suspension dose was administered through oral gavage (n=6/formulation; Dose 0.514 mg kg± 0.02 mg/kg). For oral administrations, formulation was administered using oral feeding cannula attached to 1ml syringe.

For analysis of drug concentration in plasma, blood was collected at predetermined time intervals via Retro-orbital plexus into the heparinized tubes post dose under light anesthesia. (8) The plasma was collected by centrifugation at 4000 rpm, 4ºC for 10 min.

All samples were stored at -20$^{o}$C till further analysis by using HPLC. (10) Measurements were made on 6 rats at each time point.

For analysis of drug concentration in brain homogenate, the animals were sacrificed humanely at predetermined time intervals and subsequently brain removed, washed twice with saline, made free from any adhering tissues or blood-taint and blotted up with filter paper and stored at -20$^{o}$C till further analysis by RP-HPLC. (10, 11) Measurements were made on 3 rats at each time point

## 8.13 Analysis of Blood Samples

Supernatant (plasma) was collected, stored at -20$^{\circ}$C and thawed at room temperature for further analysis. In 0.5 ml of plasma equal quantity of the organic solvent (500µL acetonitrile) was added, to precipitate the protein. Samples were centrifuged at 4 $^{o}$C; 5000 rpm for 10 min. Clear supernatant was collected and filtered through syringe filter. The samples were analyzed by RP-HPLC after suitable dilutions. The analytical method for plasma analysis was as discussed in Table 4.20.

## 8.14 Pharmacokinetic Parameter

All the pharmacokinetic parameters were calculated by non-compartmental analysis (extra vascular) using Kinetica software (version 5, Therma Scientific, Philadelphia, PA, USA) and recorded in tables 8.10 and 8.11 for ME, MME and Suspension.

## 8.15 Statistical Analysis

All data are presented ± SD, differences between three groups were analyzed by One-way ANOVA followed by post hoc Bonferroni test using graph pad prism 6. Significance was accepted at $P<0.5$ for all values.

## 8.16 Result and Discussion

### Table 8.8 Plasma Drug Concentration at Predetermined Time Intervals

| Time (min) | Plasma Drug Concentration (ng/ml) | | |
|---|---|---|---|
| | ME | MME | Suspension |
| 0 | 0 | 0 | 0 |
| 15 | 210.41 ± 11.56 | 220.64 ± 10.57 | 20.34 ± 17.64 |
| 30 | 316.203 ± 13.05 | 348.01 ± 17.64 | 109.41 ± 30.41 |
| 90 | 438.66 ± 10.49 | 468.63 ± 33.80 | 161.32 ± 13.83 |
| 240 | 391.34 ± 11.84 | 434.78 ± 26.84 | 84.43 ± 17.54 |
| 600 | 165.15 ± 15.67 | 214.96 ± 15.39 | 3.63 ± 16.49 |
| 1440 | 5.091 ± 11.682 | 8.14 ± 14.72 | - |

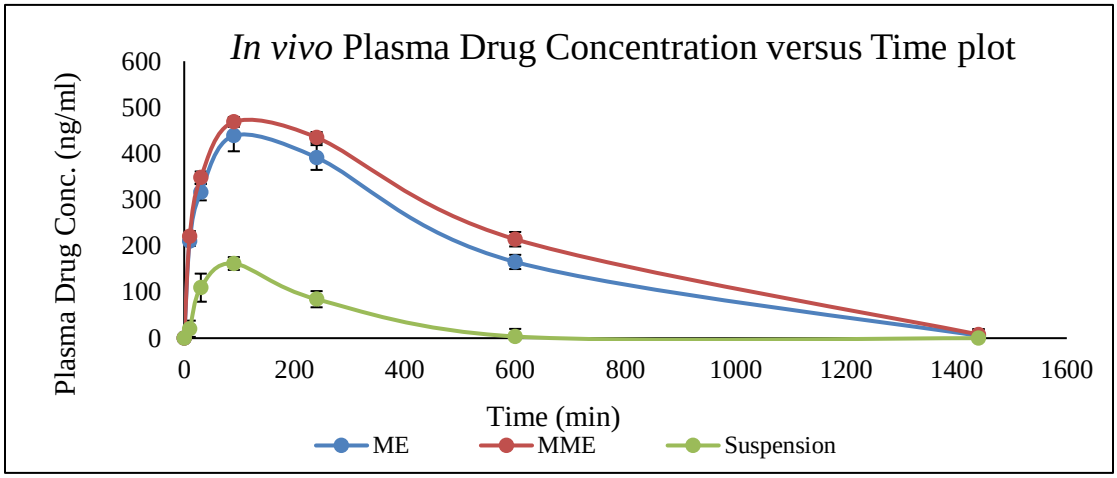


**Fig. 8.2 Plasma Drug Concentration vs Time Profiles of Vinpocetine after Nasal Administration of ME, MME and Oral Administration of Suspension.**

### Table 8.9 Brain Drug Concentration at Predetermined Time Intervals

| Time (min) | Brain Drug Concentration (ng/ml) | | |
|---|---|---|---|
| | ME | MME | Suspension |
| 0 | 0 | 0 | 0 |
| 15 | 536.87±12.21 | 433.44± 10.64 | 58.71± 10.94 |
| 30 | 654.342±8.461 | 729.15± 18.13 | 79.319± 10.54 |
| 90 | 686.33±10.34 | 770.701± 17.02 | 34.14± 11.48 |
| 240 | 457.804±11.66 | 513.46±11.74 | 9.033± 10.87 |
| 600 | 121.43±19.112 | 219.712± 10.67 | - |
| 1440 | 6.166±4.61 | 17.191± 10.22 | - |

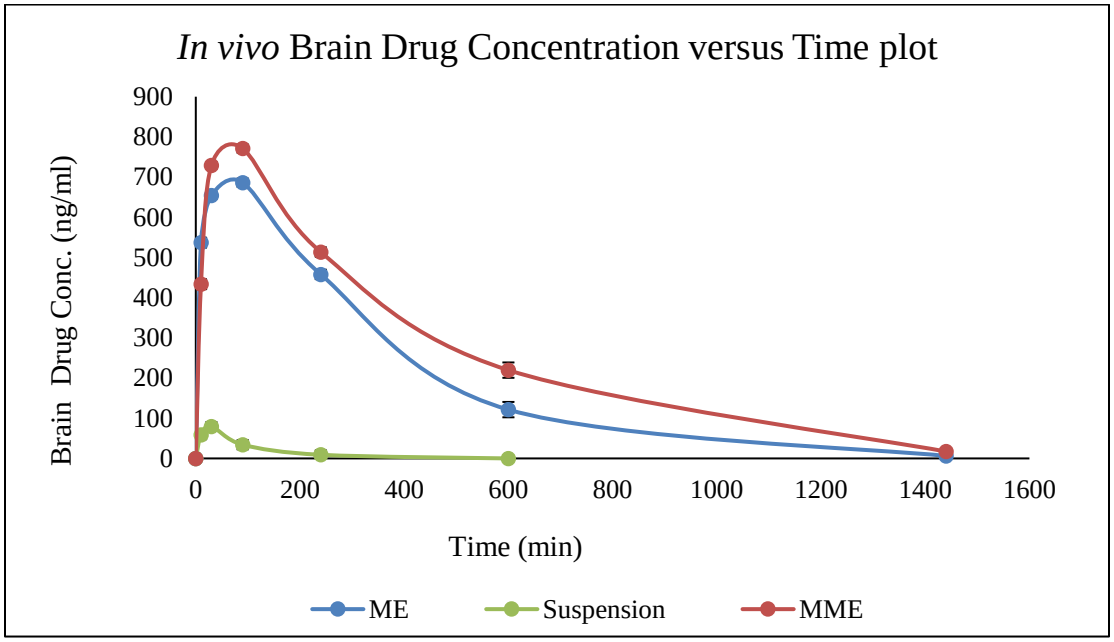


**Fig. 8.3 Brain Drug Concentration vs Time Profiles of Vinpocetine after Nasal Administration of Vinpocetine loaded ME, MME and Oral Administration of Suspension.**

**Table 8.10 Plasma Pharmacokinetic Parameters of Vinpocetine for Various Formulations after Administered (0.514 mg/kg) into Rats (n = 3, mean ± SD)**

| Pharmacokinetic Parameters | ME | MME | Suspension |
|---|---|---|---|
| Cmax (µg/ml) | 443.46 ± 12.45*** | 501.4 ± 13.78*** | 155.7 ± 24.11 |
| Tmax (hr) | 1.5 | 1.5 | 1.5 |
| $AUC_{Total}$ (ng/ml*hr) | 4373.7 ± 341.44*** | 5692.3 ± 357.02*** | 717.8 ± 17.63 |
| $AUMC_{Total}$ (ng/ml*hr) | 33844.6 ± 614.72*** | 56194.5 ± 722.81*** | 2043.9 ± 564.99 |
| $t_{1/2}$ | 5.36 ± 0.06*** | 6.74 ± 0.11*** | 1.52 ± 0.16 |
| MRT (hr) | 7.73 ± 0.13*** | 9.87 ±0.08*** | 2.84 ± 0.11 |
| Relative Bioavailability (Fr) | 6.09 | 7.93 | - |

Each point represents Mean ± SD (n=3), One-way ANOVA followed by post hoc Bonferroni test using graph pad prism 6. *p<0.5, **p<0.01, ***p<0.001 compared to suspension.

**Table 8.11 Brain Pharmacokinetic Parameters of Vinpocetine for Various Formulations after Administered (0.514 mg/kg) into Rats**

| Pharmacokinetic Parameters | ME | MME | Suspension |
|---|---|---|---|
| **Cmax (ng/ml)** | 694.13 ± 11.23*** | 767.8 ± 10.54*** | 71.6 ± 12.46 |
| **Tmax (hr)** | 1.5 | 1.5 | 0.5 |
| **$AUC_{Total}$ (ng/ml*hr)** | 4950.03 ± 347.31*** | 6181.58 ± 298.86*** | 149.67 ±12.92 |
| **$AUMC_{Total}$ (ng/ml*$hr^2$)** | 23200.5 ± 534.55*** | 41043.4 ± 476.52*** | 250.19 ± 16.63 |
| **$t_{1/2}$ (hr)** | 3.2 ± 0.06*** | 4.61 ± 0.07*** | 1.11 ± 0.17 |
| **MRT (hr)** | 4.68 ± 0.07*** | 6.64 ± 0.06*** | 1.67 ± 0.11 |
| **Relative Bioavailability (Fr)** | 33.07 | 41.30 | - |

Each point represents Mean ± SD (n=3), One-way ANOVA followed by post hoc Bonferroni test using graph pad prism 6. *p<0.5, **p<0.01, ***p<0.001 compared to suspension.

Result represented in Fig. 8.2 and Fig. 8.3, shows the plasma concentration vs time profile in rats after a single nasal administration of Vinpocetine ME, MME with compared to oral dose of Vinpocetine suspension. From the results, Vinpocetine concentrations in rat's plasma treated with ME, MME were significantly higher than those treated with suspension.

The pharmacokinetic parameters for Vinpocetine drug suspension and ME, MME are listed in Table 8.10 and 8.11. The Cmax, AUC, MRT and Relative Bio-availability (Fr) for ME, MME were significantly higher than that of drug suspension. This indicated that ME and MME achieved higher amount of drug in a shorter time period after nasal administration. Cmax for ME was about 2.8 times and for MME was about 3.2 times higher compared to that of suspension, while AUC for ME 6.1 and for MME was about 7.9 times higher compared to that of suspension. $t_{1/2}$ of ME increased by about 3.5 and of MME increase by 4.4 times higher compared to suspension shows the increased circulation time of ME and MME. This leads to increase in MRT of ME and MME by approximately 2.7 times and 3.5 times respectively to that of suspension. Increase in all the above parameters contributed to 6.1 fold and 7.9 fold surge in bioavailability of ME and MME compared to suspension. The higher Cmax was achieved with higher values of

AUC, MRT and Fr observed in ME and MME suggest improvement in the rate and extent of drug concentration in the systemic circulation.

Intranasal administration of ME and MME showed highest Cmax (brain) compared to orally administered suspension formulation. The reason is that by nasal administration first pass metabolism can be avoid. Also, the Cmax (brain) for intranasal ME and MME was found to be approximately 9.7 folds and 10.7 folds higher when compared to oral administrations of suspension respectively. AUC (brain) of intranasal ME and MME is approximately 33 and 41 folds higher than oral suspension demonstrating nasal administration of ME and MME are suitable formulation to deliver drugs effectively to the brain. AUC of MME was 1.2 times higher than that of ME represents that the drug reach to brain by MME is higher than that of ME, the reason behind that is because of mucoadhesive agent which open tight junction and give higher AUC than ME. The increased in AUC of the brain demonstrate selective nose to brain transport of drugs following intranasal delivery than oral administration.

## 8.8 References

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