## 2.1 Abstract

Cerebral ischemia affects hepatic enzymes and brain permeability extensively. Piracetam was investigated up to phase III of clinical trials, and there is a lack of data on brain penetration in cerebral ischemic condition. Thus, knowledge of the pharmacokinetics and brain penetration of piracetam during ischemic condition would aid to improve pharmacotherapeutics in ischemic stroke. Focal cerebral ischemia was induced by middle cerebral artery occlusion for 2 hr in male Wistar rats followed by reperfusion. After 24 hr of middle cerebral artery occlusion or 22 hr of reperfusion, piracetam was administered for pharmacokinetic, brain penetration, and pharmacological experiments. In a pharmacokinetic study, blood samples were collected at different time points after 200 mg/kg (oral) and 75 mg/kg (intravenous) administration of piracetam through the right external jugular vein cannulation. In brain penetration study, the cerebrospinal fluid, systemic blood, portal blood, and brain samples were collected at pre-designated time points after 200 mg/kg oral administration of piracetam. In a separate experiment, the pharmacological effect of the single oral dose of piracetam in middle cerebral artery occlusion was assessed at a dose of 200 mg/kg. All the pharmacokinetic parameters of Piracetam including area under curve ($AUC_{0-24}$), maximum plasma concentration ($C_{max}$), time to reach the maximum plasma concentration ($t_{max}$), elimination half-life ($t_{1/2}$), volume of distribution ($V_z$), total body clearance, mean residence time, and bioavailability were found to be similar in ischemic stroke condition except for brain penetration. Piracetam exposure ($AUC_{0-2}$) in brain and CSF were found to be 2.4 and 3.1 fold higher, respectively, in ischemic stroke compared to control rats. Piracetam significantly reduced infarct volume by 35.77% caused by middle cerebral artery occlusion. There was no change in the pharmacokinetic parameters of piracetam in the ischemic stroke

model except for brain penetration. This indicates that variables influencing brain penetration may not be limiting factors for use of piracetam in ischemic stroke.

Keywords:

Focal cerebral ischemic stroke, animal model, middle cerebral artery occlusion, pharmacokinetics and brain penetration.

**2.2 Introduction**

Stroke and transient ischemic attack are the common causes of death and dementia in developed countries. However, ischemic stroke accounts for approximately 80% population of all strokes and is caused by sudden cessation of blood flow to the brain by the formation of embolism or thrombosis in cerebral blood vessels (Deng et al. 2016 and De et al. 1999). Recombinant tissue plasminogen activator (rtPA) is the only drug which is effective in the treatment of medical emergency of acute ischemic stroke (Tsai et al. 2015). However, the therapeutic window of rtPA action is up to 4.5 h after stroke, and therefore, rtPA is applicable as a treatment in only up to 5% of all patients (Tsai et al. 2015). Thus, there is a pressing need for other, more widely applicable, treatment options. Clinical studies have shown that piracetam treatment significantly improves cognitive changes after ischemic stroke (Chen et al. 1997; Stahlhut et al. 2014; Yeo et al. 2017; Platt et al. 1993 and Grotemeyer et al. 2000). ADP (adenosine diphosphate) is a molecule involved in the activation of the platelets (Brass et al. 2003) and leads to vascular occlusion resulting in oxidative stress, inflammation, and cell death, which are the key contributory pathways underlying lesion progression in ischemic stroke. A balance between released of thromboxane from the platelets and prostacyclin from the vessel wall controls thrombus formation (Winnicka et al. 2005). Piracetam inhibits thromboxane and also has direct effect

on the vascular wall to stimulate prostacyclin production (Winnicka et al. 2005). Piracetam slows down brain aging, and improves cerebral blood flow and oxygen availability in the brain (Sara et al. 1972; Moldavkin et al. 2005 and Nalbandian et al. 1983). It is also reported to be helpful in the treatment of Alzheimer's disease, dyslexia, and dementia (Tripathi et al. 2017 and Wang et al. 2010). Piracetam has shown neuroprotective effect in the early therapy of acute hemispheric stroke (Wustmann et al. 1981; Depresseux et al. 1986 and De et al. 1997), aphasia (Enderby et al. 1994 and Huber et al. 1999) and palatal myoclonus (Karacostas et al. 1999). In spite of its potential, piracetam was investigated only up to phase III of clinical trials for stroke treatment (Saletu et al. 1995 and Savitz et al. 2007).

Blood-brain barrier (BBB) disruption has been observed in ischemic stroke from 24 hr (Pillai et al. 2009) to days (Durukan et al. 2009) or weeks (Strbian et al. 2008). In stroke, BBB disruption is believed to be biphasic which potentially limits the therapeutic time window and may alter the brain exposure of a compound (Kuroiwa et al. 1985 and Belayev et al. 1996). Piracetam improved recovery from stroke (Platt et al. 1993 and Grotemeyer et al. 2000; Yeo et al. 2017) and we were interested to know the brain penetration of piracetam during ischemic stroke in-order to determine the access of piracetam to brain tissue in focal ischemia condition which could be one of the critical factors influencing the pharmacological action of piracetam (Savitz et al. 2007; Pillai et al. 2009 and Latour et al. 2004). Further, most of the drugs are metabolized by liver cytochrome P450 (CYP 450) enzymes, earlier studies has shown increase in half-life and mean residence time of some compounds during cerebral ischemic-reperfusion injury. Cerebral ischemic-reperfusion injury is reported to down regulate CYP 450 enzymes (Zhu et al. 2013).Thus, pharmacokinetic study including brain penetration in cerebral ischemic condition was done to understand the

pharmacotherapeutics of piracetam under the pathological condition of cerebral ischemic-reperfusion injury after 24 hr of middle cerebral artery occlusion.

The pharmacokinetics of piracetam has been reported (Doheny et al. 1996 and Barkat et al. 2014). After oral administration, it has fast and complete absorption with peak plasma level at 30-90 min (Sahu et al. 2013). It crosses blood-brain barrier (BBB) but at slower rate due to high hydrophilicity of cerebrospinal fluid (Calliauw et al. 1975 and Winblad et al. 2005). The drug and its fraction move out by excretion through urine after 30 h, oral administration (Tacconi et al. 1985 and Gouliaev et al. 1994). Piracetam is not metabolized in liver and also not bound to plasma protein albumin with plasma half-life of 5 h and cerebrospinal fluid (CSF) half-life of 7.7 h (Gobert et al. 1977 and Brown et al. 1993). The oral bioavailability is almost close to 100% and excreted unchanged in urine while 1-2% in feces (Tacconi et al. 1985 and Gouliaev et al. 1994). However, there are no reports describing the pharmacokinetic and brain penetration of piracetam during the pathological condition of ischemic stroke. Therefore, it is important to determine the pharmacokinetics including brain penetration of drug in pathological condition of stroke to understand the therapeutic effect in context of underlying pathophysiology. Middle cerebral artery occlusion model mimics the human stroke condition and mostly used in studies related to ischemic stroke (Longa et al. 1989).

Therefore, in the present study, we have used middle cerebral artery occlusion model to induce focal cerebral ischemia. New guidelines on treating stroke suggest that more people could be eligible for life-saving clot removal and treatments, expanding the "golden window" when doctors can minimize or prevent permanent damage from six to 24 hr. Bansal et al has demonstrated that the administration of anti-platelet drugs should be administered after 24 of

ischemia, showed anti ischemic effect (Bansal et al., 2013). Piracetam improved outcome relative to placebo in acute stroke in which high-dose piracetam was given intravenously 3-5 days after the onset of stroke (Platt et al 1993; Herrschaft H 1988). In contrast, some literature shows piracetam did not influence the stroke outcome when given within 12 hr of the onset (De et al 1997) and has weaker efficacy when compared to aspirin in the prevention of secondary stroke. Perhaps therapeutic window play an important part in the effect of piracetam on MCAO model. Thus, we believed that piracetam weaker efficacy is may be due to its poor brain penetration as BBB leakage increases slowly following 2 hr of middle cerebral artery occlusion, which peaks at 24 hr (Pillai et al. 2009). Therefore, in the present study, we have evaluated the brain penetration of piracetam 24 hr post stroke.

### 2.3 Hypothesis

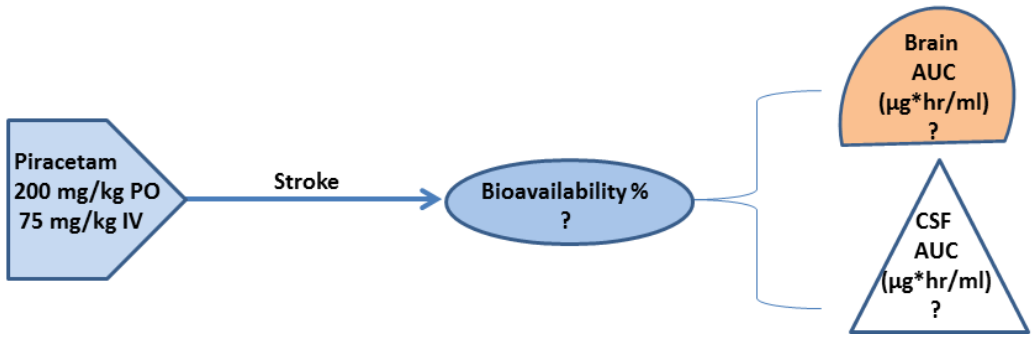


Figure 2.1 Proposed hypothesis

### 2.4 Materials and methods

#### 2.4.1 Chemicals

Piracetam (active pharmaceutical ingredient) was obtained as gift from UCB India Pvt. Ltd., Mumbai, India. Its chemical structure is shown in Fig. 2.2. Methanol, perchloric acid, and sodium heparin were purchased from Sigma Chemicals, India.

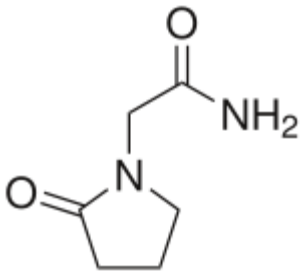


Figure 2.2 Chemical structure of piracetam

### 2.4.2 Animals

Adult male albino Wistar rats 250-280 g were procured from the Central Animal House, IMS (Institute of medical Sciences), Banaras Hindu University (BHU). The animals were acclimatized for 7 days at 25 ± 1 °C with a 12-hr light-dark cycle and were allowed free access to food (Amrut Laboratory Animal feed, Sangli, India) and water throughout the experiment. All efforts were made to minimize the number of animals used and all experiments were conducted in accordance with the principles of laboratory animal care (National Research Council, US Committee for the Update of the Guide for the Care and Use of Laboratory Animals 2011) guidelines. Prior to experiment on the animal, an approval from animal ethics committee was granted (date of the approval 03-10-2015, Ref No. Dean/2015/CAEC/1421) and all surgical procedures were conducted under aseptic conditions. The experiments were done in three sets of animals. First (pharmacokinetic) and second sets (brain penetration) of experiments consisted of two groups (control and ischemic group) of five animals each. Third set (pharmacology) of the experiment consists of three groups (control, ischemic, and vehicle group) of five animals each. In the control group, surgery was performed using sodium pentobarbitone (45 mg/kg; i.p.) anaesthesia to isolate the arteries; however, the filament was not inserted into the middle cerebral artery. The

ischemic stroke group and vehicle group consisted of animals which underwent filament occlusion (middle cerebral artery occlusion). Piracetam was administered to the control and ischemic groups, whereas to the vehicle group, distilled water was administered.

### 2.4.3 Experimental design

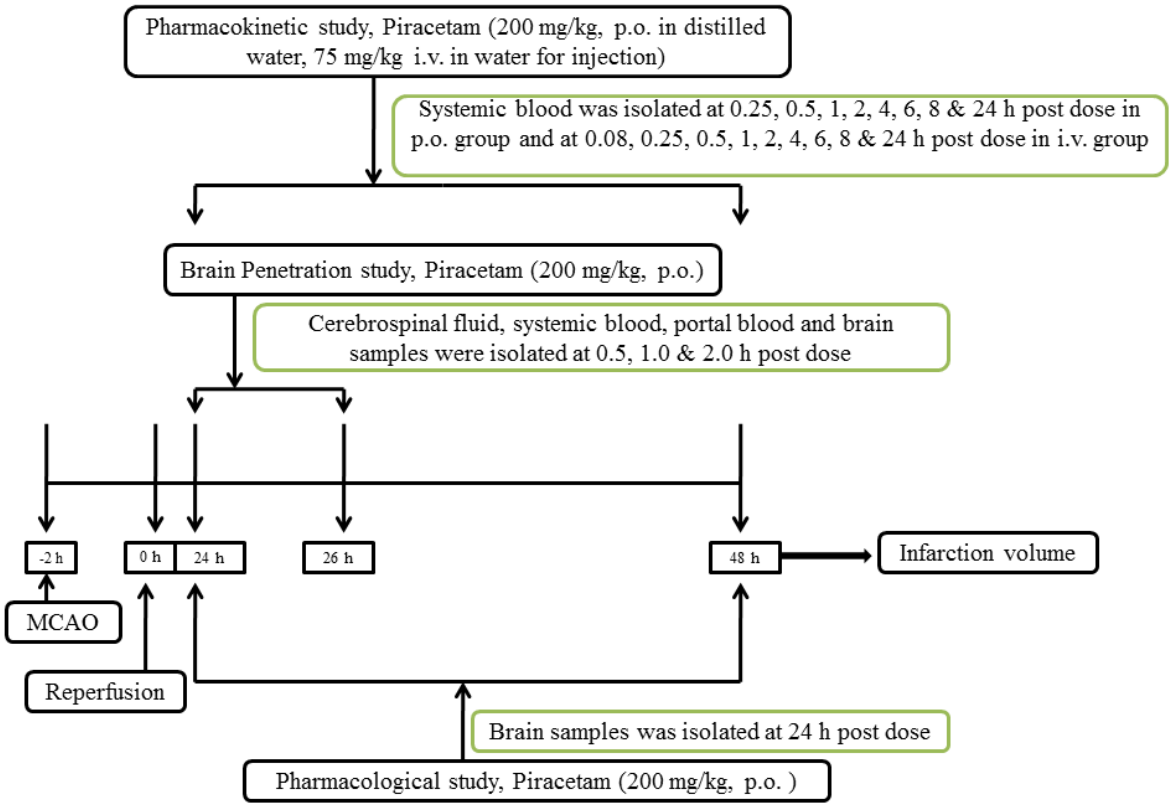


Figure 2.3 Schematic representation of the experimental design where p.o: per-oral, i.v.: intravenous, h: hour, MCAO: middle cerebral artery occlusion and mg/kg: milligram/kilogram.

### 2.4.4 Pharmacokinetic and Brain Penetration Studies

Pharmacokinetic study was performed after 24 hr of middle cerebral artery occlusion (Paliwal t al 2018). For oral and intravenous administration, piracetam was dissolved in distilled water and water for injection, respectively. The resulting solution was vortexed

followed by sonication for 30 s. Piracetam was administered orally in 12 hr fasted rats, whereas it was administered intravenously in non-fasted rats. Rats were dosed at 200 mg/kg orally (10 mL/kg dose volume, 20 mg/mL strength) through gavage and 75 mg/kg intravenously (2 mL/kg dose volume and 37.5 mg/mL strength) through the tail vein. The dose was selected based on an earlier study on the effect of piracetam in neuroinflammation (Tripathi et al. 2017). Blood samples of 0.25 mL were collected at 0.25, 0.5, 1, 2,4, 6, 8, and 24 hr after oral, at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8,and 24 hr after intravenous administration through jugular vein cannula. Sampling was done in all animals at these time points. Blood samples were transferred to pre-labelled heparin-coated sampling tubes at the respective time points. An equal volume of saline was replaced after each blood withdrawal. Plasma samples were stored at -80°C until analysis (Muddana et al. 2014). Rats were killed by cervical dislocation after completion of the study. The brain penetration study was conducted at 200 mg/kg orally (10 mL/kg dose volume and 20 mg/mL strength) in non-fasted rats. CSF, cardiac blood, portal blood, and brain samples were collected (N = 5/time point) at 0.5, 1,and 2h postdose. The isolated brain was splashed with water to remove traces of blood present on it. Four vols (weight/volume) of ice-cold water were added and homogenized to obtain 20% brain homogenate on this whole brain tissue. The concentration of piracetam in the brain sample was obtained after multiplying 20% brain homogenate value by the homogenization factor (Nirogi et al. 2013).

### 2.4.5 Infarct Volume Measurement

Piracetam 200 mg/kg orally was administered after 24 hr of middle cerebral artery occlusion or 22 hr of reperfusion. The rats were euthanized and brain tissue was collected 24 hr postdose. Rat brains of each group were frozen at -20 °C for 5 min and sectioned into 2 mm

thick coronal slices. Slices were incubated for 20 min at 37 °C in 2% TTC solution in phosphate buffer saline (PBS, pH 7.4). Color images of these slices were captured, and the size of infarction was calculated using ImageJ software. To obtain the total infarct area, infarcted areas of all sections were added. Further, brain oedema may impair the exact infarction volume; thus, the obtained infarct area needs to be corrected. The corrected infarct volume was calculated as follows: corrected infarct area = measured infarct area multiplied by {1 - [(ipsilateral hemisphere area-contralateral hemisphere area)/contralateral hemisphere]}. To determine the infarct volume, total infarct areas were multiplied by the thickness of the brain sections (Berti et al. 2002 and Wu et al. 2013).

### 2.4.6 Jugular Vein Cannulation

Jugular vein cannulation was performed for the collection of the blood from the rat. The male Wistar rats were anaesthetized using pentobarbitone sodium 40 mg/kg i.p. Once deep anaesthesia was achieved, rats were transferred to the surgery table, and body temperature was maintained at 37 ± 0.5 °C. A small incision was made near to right arm, and a jugular vein cannula (PE 50 tube OD 0.95 mm, ID 0.56 mm) was inserted 3.5 cm apart into the right external jugular vein. The jugular vein cannulation was tunnelled beneath the skin and exteriorized through a small stab wound in the back of the neck. When not in use, the catheter was flushed with 50-IU heparin/mL of 0.9% saline. The incision was sutured, and betadine was applied over the incision. The rats were monitored until they regained their righting reflex. The animals were allowed to recover at least for 24-hr, after jugular vein catheterization before the pharmacokinetic study (Muddana et al. 2015).

### 2.4.7 Middle Cerebral Artery (MCA) Occlusion

Middle cerebral artery occlusion method with the modified intraluminal technique was used to produce focal ischemia (Longa et al. 1989). Rats were anaesthetized by the administration of 40-mg/kg sodium pentobarbitone i.p. and then transferred to the surgical table with a heating lamp to maintain a constant body temperature of 37 ± 0.5 °C. Rat was placed in a supine position with forelimbs fixed on the table bytape, and the fur on the ventral neck was shaved, and the skin was cleaned with 0.5% betadine and 75% alcohol. Muscle fascia was separated further to expose the left common carotid artery. Muscle fascia was separated further to expose the external carotid artery and internal carotid artery. A 5.0-cm-length 3-0 monofilament suture was introduced into the carotid artery lumen through a small nick and gently pushed from the internal carotid artery lumen to block the origin point of the middle cerebral artery. Approximately 18-22 mm length of nylon filament was inserted to reach the MCA blockade site from the bifurcation point. The external carotid artery stump was clamped around the intraluminal nylon suture to prevent bleeding. Reperfusion was done by gently removing the filament after 2 hr of ischemia. Animals were allowed to recover from the anaesthesia and, on regaining the righting reflex, were transferred to polypropylene cages in the animal room (Longa et al. 1989). A mortality rate of about 10 % was observed after two hours of occlusion, followed by 24 hr of reperfusion. The animal which died during the experiment were not included in the experimental study groups.

### 2.4.8 Quantification of Piracetam

A stock solution was prepared by dissolving and sonicating 200 mg of piracetam in 100-mL distilled water. The stock solution was used to prepare six standard working solutions, with concentrations ranging from 1 to 1000 µg/mL. All these stock and standard working

solutions were prepared freshly, on a regular daily basis. The six standards samples with a concentration range from 0.05 to 500 µg/mL were obtained by adding an appropriate amount of the working solution to plasma, CSF, and blank brain samples. Then, the mixtures were shaken with a vortex mixer for 15 s. The accuracy and precision of the analytical method were evaluated using quality control samples obtained from the following concentrations of piracetam: A = 0.05 µg/mL, B = 5.0 µg/mL, and C = 500 µg/mL. The lowest detected concentration was obtained when the accuracy was within ± 20%, and the precision was below 15%; known as a lower limit of quantitation (LLOQ) (Barkat et al. 2014 and Curticapean et al. 2007).

Aliquots of 100 µL of the samples were placed in a microcentrifuge tube, and 15 µL of 20% perchloric acid were added. After that, the mixtures were shaken in the vortex mixer for 30 s and then centrifuged for 10 min at 13,000 rpm. The obtained supernatant was transferred into chromatographic vials, from where 20 µL of the solution was injected into the column (Nirogi et al. 2013). The analyses were carried out isocratically in an HPLC system consisting of a Kontron Dionex LC pump (LPG-3400A), Kontron Dionex ultraviolet detector (UVD 340U). The chromatography was performed using symmetry C18 (4.6 9 250 mm) column at 30 °C temperature. The mobile phase consisted of an acetonitrile-1% formic acid in water (10:90 v/v) (Wang et al. 2010). The flow rate was 1 mL/min. A 20-µL volume of the prepared sample solution was used for each injection, piracetam being detected at 205 nm (Barkat et al. 2014). The lower limit of quantitation of piracetam in plasma, CSF, and brain was found to be 0.05 µg/mL.

### 2.4.9 Data Analysis

Pharmacokinetic parameters and brain penetration data are expressed as mean ± standard error mean (SEM). Maximum plasma concentration ($C_{max}$) and time to reach the maximum plasma concentration ($T_{max}$) values were obtained directly from plasma concentration-time curves of piracetam and other pharmacokinetic parameters such as the area under the curve from time zero to 24 hr ($AUC_{0-24}$), mean residence time ($MRT_{last}$), total body clearance (Cl), the volume of distribution ($V_z$), and elimination half-life ($t_{1/2}$) were calculated by noncompartmental methods using software PK Solver. Statistical comparisons among control and ischemic stroke groups were performed with Graph-Pad Prism version 5 (San Diego, CA) using two-way analysis of variance (ANOVA) followed by Bonferroni posttests and unpaired t-test. A level of $p<0.05$ was accepted as statistically significant.

## 2.5 Results

### 2.5.1 Effect of Cerebral Ischemic-Reperfusion Injury on Pharmacokinetics of Piracetam

Plasma drug log concentration-time curves of piracetam following oral (200 mg/kg) and intravenous (75 mg/kg) administration to control and ischemic stroke rats is shown in Figs. 2.4 and 2.5, respectively. Two-way ANOVA showed that the concentration-time profile of piracetam given through oral route was similar in control and ischemic groups except for 0.25, 1.0, and 2.0 h time points. However, the concentration-time profile of piracetam given through the intravenous route was similar in all time points. Two-way ANOVA showed that there were no significant differences in the pharmacokinetic parameters, summarized in Table 2.1, during ischemic stroke.

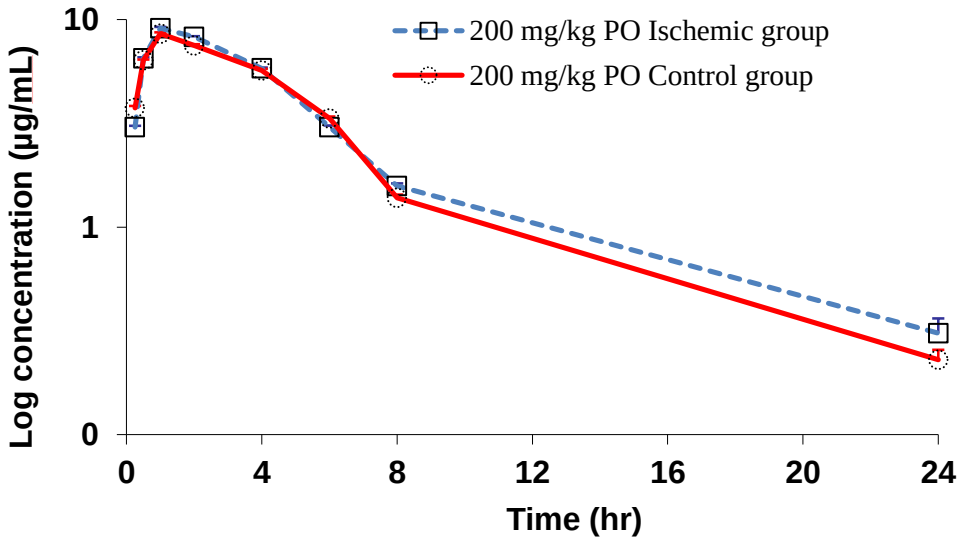


Figure 2.4 Plasma log concentration-time curve of piracetam in control group rats and ischemic stroke group rats after oral administration of piracetam (200 mg/kg). Data are expressed as mean ± SEM (N=5).

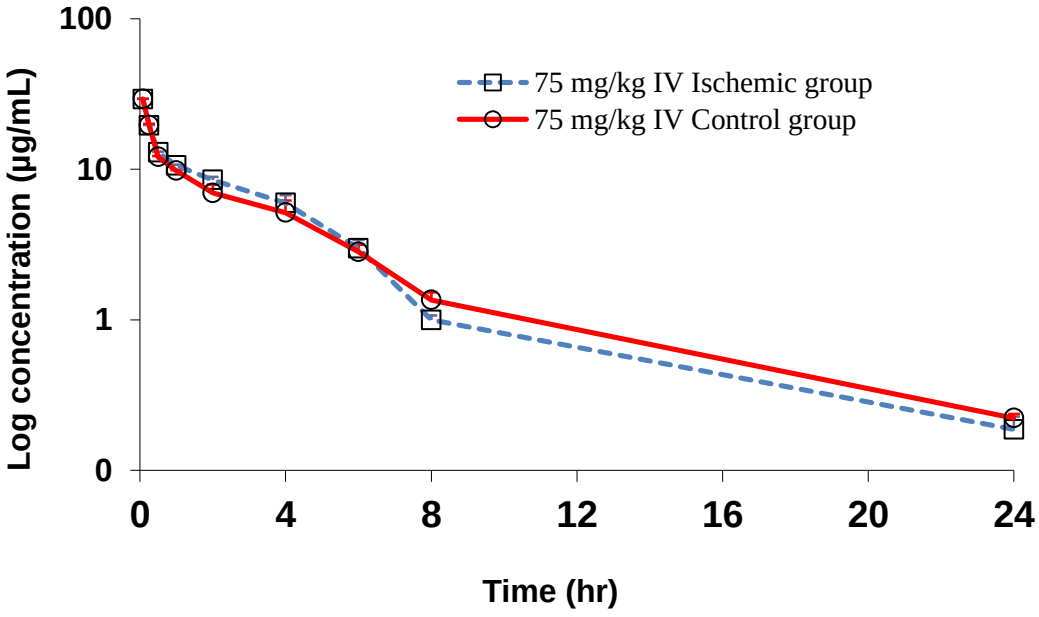


Figure.2.5 Plasma log concentration-time curve of piracetam in control group rats and ischemic group rats after intravenous administration of piracetam (75 mg/kg). Data are expressed as mean ± SEM (N=5).

**Table 2.1: Pharmacokinetic parameters of piracetam in control and ischemic stroke rats**

| PK Parameters | Control group | | Ischemic group | |
|---|---|---|---|---|
| | **p.o.** | **i.v.** | **p.o.** | **i.v.** |
| Dose (mg/kg) | 200.00 | 75.00 | 200.00 | 75.00 |
| $C_{max}$ (µg/mL) | 8.56 ± 0.62 | - | 9.08 ± 0.66 | - |
| $t_{max}$ (h) | 1.00 ± 0.00 | - | 1.00 ± 0.00 | - |
| $AUC_{0-24h}$ (µg.h/mL) | 53.37 ± 2.59 | 61.47 ± 2.71 | 56.76 ± 2.54 | 63.10± 2.93 |
| $t_{1/2}$ (h) | - | 4.85 ± 0.39 | - | 4.46 ± 0.63 |
| $MRT_{last}$ (h) | 5.54 ± 0.20 | 4.69 ± 0.10 | 6.27 ± 0.41 | 4.18 ± 0.27 |
| $V_Z$ (L/kg) | - | 1.16 ± 0.11 | - | 1.20 ± 0.04 |
| Cl (L/kg/h) | - | 8.27 ± 0.95 | - | 8.03 ± 1.18 |
| Bioavailability (%) | 32.56 ± 1.82 | | 33.74 ± 1.97 | |

PK: Pharmacokinetic; p.o.: per-oral, i.v.: intravenous; $AUC_{0-24}$: area under the curve from time zero to twenty-four hours; $C_{max}$: maximum plasma concentration; $t_{max}$: time to reach the maximum plasma concentration; $t_{1/2}$: elimination half-life; $V_z$: volume of distribution; Cl: total body clearance; MRT: mean residence time.

Units: mg/kg = milligram/kilogram; µg/mL = microgram/milliliter; h = hour; µg.h/mL = microgram*hour/ milliliter; L/kg = liter/kilogram; L/kg/h = liter/kilogram/hour

Oral administration of piracetam was performed in 12 hr fasted rats.

Data are expressed as mean ± SEM (N=5). No significant difference was found against control group:P>0.05, versus the control group (unpaired *t*-test)

### 2.5.2 Effect of Cerebral Ischemic-Reperfusion Injury on Brain Exposure of Piracetam

The degree of drug uptake from plasma into brain tissue was estimated from the ratio of concentration in brain homogenate over the plasma concentration ($C_b/C_p$). Piracetam concentration in the brain, CSF, and $C_b/C_p$ was found to be increased two to three-fold at all the three-time points in ischemic stroke rats compared to control rats, as shown in Table 2.2. Furthermore, piracetam exposure ($AUC_{0-2}$) in CSF was 3.1 fold higher and in the brain was 2.4 fold higher in ischemic stroke rats compared to control rats, as shown in Table 2.3.

**Table 2.2: Brain Penetration data of piracetam in control and ischemic stroke rats after oral administration at 200 mg/kg dose**

| Time (h) | Groups | CSF (µg/ml) | Brain (µg/ml) | Portal plasma (µg/ml) | Sys. plasma© (µg/ml) | Sys. plasma® (µg/ml) | Sys.©/ Sys.® | $C_{brain}/C_{Sys..}$® ($C_b/C_p$) | $C_{portal}/C_{sys.}$® |
|---|---|---|---|---|---|---|---|---|---|
| 0.5 | Control | 0.40±0.02 | 10.42±0.62 | 13.35±0.27 | 6.37±0.32 | 7.40±0.45 | 0.85±0.02 | 1.40±0.05 | 1.82±0.08 |
| | Stroke | 1.27±0.07* | 27.54±1.56* | 13.46±0.36 | 6.51±0.43 | 6.46±0.57 | 1.01±0.04 | 4.23±0.27* | 2.04±0.07 |
| 1.0 | Control | 1.34±0.10 | 12.43±1.17 | 16.76±0.34 | 8.56±0.37 | 9.56±0.62 | 0.91±0.06 | 1.31±0.02 | 1.77±0.09 |
| | Stroke | 4.25±0.15* | 32.72±1.34* | 17.00±0.43 | 9.08±0.61 | 8.08±0.56 | 1.13±0.09 | 4.07±0.19* | 2.09±0.08 |
| 2.0 | Control | 2.64±0.12 | 13.54±0.73 | 24.27±0.83 | 7.51±0.56 | 8.51±0.50 | 0.87±0.07 | 1.58±0.03 | 2.89±0.11 |
| | Stroke | 8.01±0.44* | 34.45±1.16* | 25.01±1.10 | 8.26±0.70 | 7.66±0.31 | 1.06±0.08 | 4.51±0.12* | 3.21±0.10 |

CSF: Cerebrospinal fluid; $C_b/C_p$: Brain concentration/Plasma concentration; Sys. : Systemic; $C_{portal}/C_{sys}$: Portal plasma concentration/systemic plasma concentration

Units: µg/mL = microgram/milliliter; h = hour

® indicate systemic plasma concentration of piracetam in non-fasted rat of BP study

© indicate systemic plasma concentration of piracetam in fasted rat of PK study

Data are expressed as mean ± SEM (N=5). *P<0.05, versus control group (Two-way ANOVA followed by Bonferroni Post-hoc test)

**Table 2.3: Exposure of Piracetam in control and ischemic stroke rats at 200 mg/kg oral and 75 mg/kg intravenous doses**

| Parameters | Control group | Ischemic group |
|---|---|---|
| $AUC_{0\text{-}2hr\ IV\ systemic\ plasma}$ | 20.59±0.52 | 22.23±0.56 |
| $AUC_{0\text{-}2hr\ Portal\ plasma}$ | 31.38±0.35 | 31.97±0.21 |
| $AUC_{0\text{-}2hr\ Systemic\ plasma}$ | 15.12±0.22 | 12.48±0.20 |
| $AUC_{0\text{-}2hr\ CSF}$ | 2.53±0.12 | 7.82±0.29* |
| $AUC_{0\text{-}2hr\ Brain}$ | 21.32±0.28 | 51.27±0.26* |
| %Intestinal extraction | 43.12±1.34 | 47.92±1.56 |
| %Portal availability | 57.24±2.13 | 52.48±1.45 |
| %Hepatic extraction | 24.28±1.27 | 18.46±1.94 |

PK: pharmacokinetic; IV: intravenous (bolus); BP: brain penetration; CSF: cerebrospinal fluid; $AUC_{0\text{-}2hr}$: area under curve from time zero to two hours

Units: µg.h/mL = microgram*hour/ milliliter

Data are expressed as mean ± SEM (N=5). $^{*}P<0.05$, versus control group (unpaired *t* test)

### 2.5.3 Effect of Cerebral Ischemic-Reperfusion Injury on First-Pass Metabolism of Piracetam

First-pass metabolism of piracetam was estimated from the ratio of piracetam concentration in portal plasma over the piracetam concentration in systemic plasma ($C_{portal}/C_{systemic}$). The mean $C_{portal}/C_{systemic}$ values of piracetam in control and ischemic stroke rats at 0.5, 1, and 2 hr, time points at 200 mg/kg, p.o. dose are shown in Table 2.2. Statistical analysis by two-way ANOVA revealed that there were no significant differences between control and ischemic stroke group in $C_{portal}/C_{systemic}$ after 0.5 h postdose of piracetam. Further the intestinal extraction, portal availability and hepatic extraction data of piracetam after 200 mg/kg oral

and 75 mg/kg intravenous administration in control rats were found to be not statistically different compared to ischemic stroke rats, as shown in Table 2.3 by unpaired t-test.

### 2.5.4 Effect of Food in Pathological Condition of Cerebral Ischemia

Effect of food on piracetam exposure was estimated from the ratio of concentration in fasted plasma (pharmacokinetic study) over the non-fasted plasma (brain penetration study) at 1.0h postdose ($t_{max}$). FDA recommends no food effect if 90% confidence intervals of nonfasted/fasted $C_{max}$ ratio is within 80-125% (Cder et al. 2002). Since at 1h post-dose, the concentration ratio of piracetam was found to be in the range of 80-125%. Therefore, we considered no significant effect of food on piracetam exposure during the pathological condition of cerebral ischemia.

### 2.5.5 Pharmacological Effect of a Single Oral Dose of Piracetam

A single oral dose of piracetam significantly reduced infarct volume by 35.77% (from 27.4 to 17.6%) compared to the vehicle group ($p<0.05$). One-way analysis of variance followed by Bonferroni posttests revealed a significantly smaller infarct volume in piracetam group in contrast to the vehicle [$F(2, 14) = 94.49$; $p<0.05$], as shown in Fig. 2.6 (B).

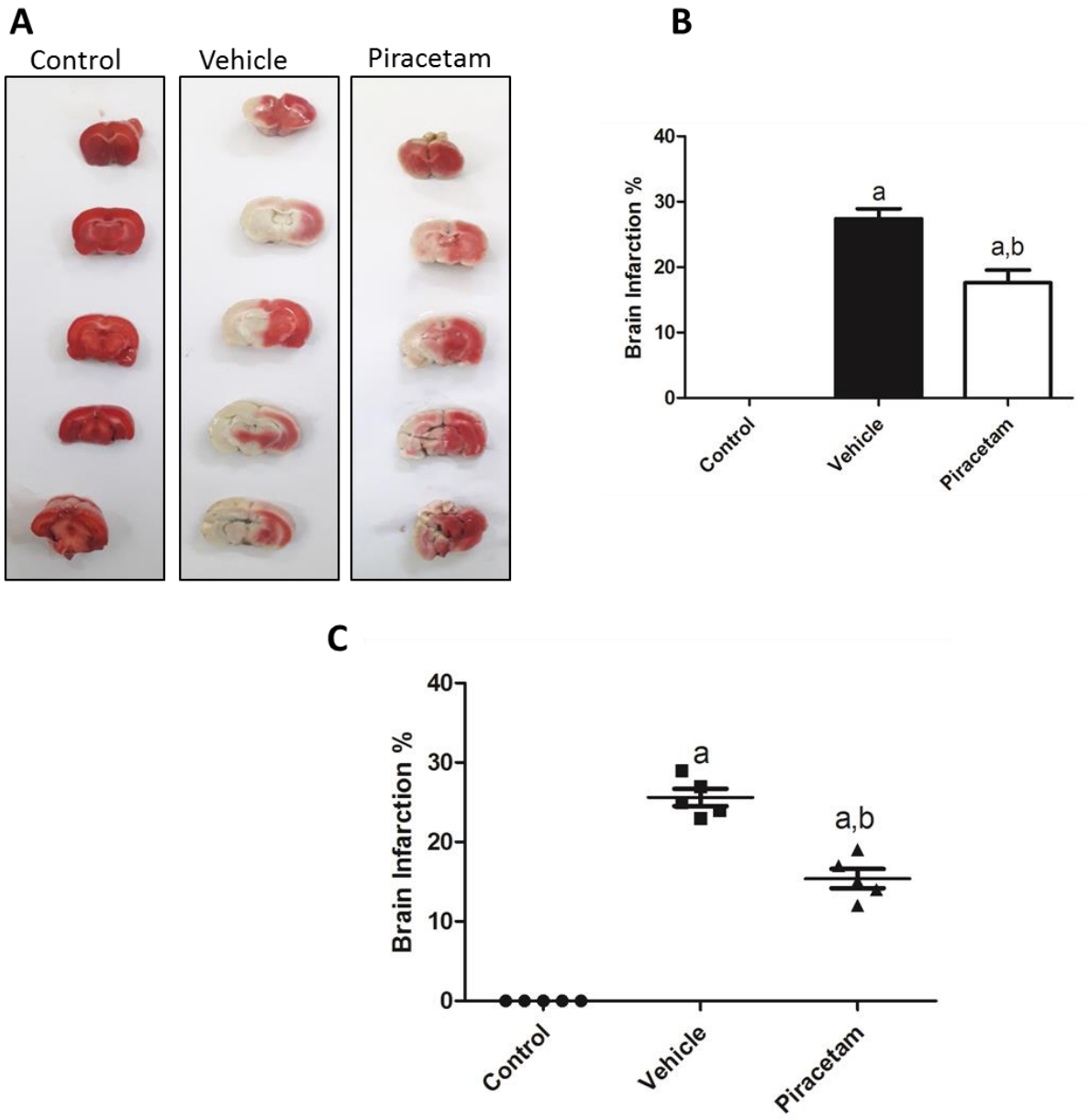


Figure 2.6 Represent the effects of piracetam on infarct volume in middle cerebral artery occlusion rats after 24 hr post-dose; (A) represents the stained images for groups, (B) and (C) represent the BARS and dot plot respectively. All values are mean ± SEM (N = 5). [a]P<0.05 compared to control group, [b]P<0.05 compared to vehicle (One-way ANOVA followed by Student Newman-Keuls post-hoc test)

## 2.6 Discussion

The salient results of the present study are that $C_b/C_p$, CSF, and brain exposure of piracetam in ischemic stroke rats were increased two to threefold, indicating a significant CNS penetration of piracetam in stroke. Piracetam significantly reduced infarct volume caused by middle cerebral artery occlusion. The pharmacokinetic parameters including first-pass metabolism and food effect of piracetam in both ischemic stroke and control rats were similar, indicating no change in the absorption, distribution, metabolism, and excretion profile of piracetam during ischemic stroke.

Our experiment shows that piracetam reaches brain tissue extensively after experimental stroke. The probable cause could be the disruption of the BBB after experimental transient focal cerebral ischemia. The disruption of the BBB has been observed from 24 hr (Pillai et al. 2009) to days (Durukan et al. 2009) or weeks (Strbian et al. 2008). Magnetic resonance imaging of stroke patients revealed that the BBB is disrupted early after stroke onset (estimated median onset time of 3.8 h) and is related with poor outcome (Latouret al. 2004 and Warach et al. 2004). In a stroke, BBB disruption is believed to be biphasic, which potentially limits the therapeutic time window and may alter the brain exposure of a compound (Kuroiwa et al. 1985 and Belayev et al. 1996). However, the exact mechanism of BBB disruption is yet to be identified. These works, coupled with the present data, demonstrate that limited access of drugs to brain tissue may not be a critical factor in focal ischemia as the exposure is high due to pathological changes in the BBB. Since piracetam brain exposure, $C_b/C_p$, and CSF exposure significantly increased after cerebral ischemic-reperfusion injury, it could be due to breakdown of BBB. The importance of brain penetration of piracetam could have been further validated by the use of another drug which

is hydrophilic. However, as the study also correlated pharmacokinetics with a pharmacological effect, the choice of such drug is limited as most of the first-line drugs used for ischemia is lipophilic in nature. A single dose of piracetam given 200 mg/kg p.o. showed two to threefold more CNS exposure in middle cerebral artery occlusion rats and was effective in reducing infarct volume caused by middle cerebral artery occlusion. Thus, brain penetration of the piracetam may not be a limiting factor for the pharmacological activity of piracetam in middle cerebral artery occlusion model. There were two to threefold increase in drug concentration in CNS compared to plasma up to 2 hr. This observation indicates an opportunity to reduce the dose of piracetam up to two to threefold for therapeutic efficacy. However, as the LD-50 of piracetam is 5600 mg/kg (oral) in rats, and safe clinically up to 24 g/day, there may not be any significant toxic effects at the concentration used in the experiment (De et al. 1999).

Most of the drugs are metabolized by liver cytochrome P450 (CYP 450) enzymes, earlier studies have shown an increase in half-life and mean residence time of some compounds during cerebral ischemic-reperfusion injury. Cerebral ischemic-reperfusion injury is reported to down-regulate CYP 450 enzymes (Zhu et al. 2013). The extent of the first-pass metabolism by the liver after oral administration of piracetam was estimated from the ratio of concentration in portal plasma over the systemic plasma concentration that is $C_{portal}/C_{systemic}$, which was found to be similar in ischemic stroke and control rats at all the three-time points. Furthermore, no significant change in hepatic metabolism was found. Thus, there is no change in in-vivo metabolism of piracetam after cerebral ischemic injury, even though studies have reported altered hepatic function in cerebral ischemic-reperfusion injury (Tacconi et al. 1985 and Gobert et al. 1977). Metabolite measurement and renal clearance are

the additional parameters which are important when there is a drastic change in the metabolism and excretion of the drug. However, in the present study, the pharmacokinetic parameters, including half-life, mean residence time, total body clearance, and first-pass metabolism, were found to be similar in both stroke and control rats. Furthermore, piracetam is not actively metabolized in the liver (Gobert et al. 1977 and Brown et al. 1993) and mostly excreted unchanged in urine (Tacconi et al. 1985 and Gouliaev et al. 1994). Therefore, there may not be significant changes in the metabolism and excretion profile of piracetam during ischemia.

The observed similarity in piracetam plasma $AUC_{0-24}$ in ischemic stroke and control rats indicates no change in gastrointestinal absorption of piracetam after cerebral ischemic-reperfusion injury, which was further confirmed by similar $C_{max}$ and $t_{max}$. Furthermore, the portal availability of piracetam after ischemic-reperfusion injury remains the same. Both these points indicate that cerebral ischemic injury does not affect intestinal extraction and absorption of piracetam. Degree of distribution of the drug in body tissue was evaluated by $V_z$ which was found to be similar in ischemic stroke and control rats. This shows that the distribution of piracetam remains the same after the ischemic-reperfusion injury. Earlier studies have been reported that piracetam was wholly and readily absorbed after oral administration (Sahu et al. 2013), and therefore, the presence of food does not significantly affect its absorption. This is one of the reasons for the absence of a significant effect of food on the pharmacokinetics of piracetam in ischemic reperfused rats. Furthermore, there were no significant differences in the pharmacokinetic parameters which were determined after oral and intravenous administration in ischemic-reperfused and control rats; in spite of the fact that pharmacokinetic profile of drug may be altered during cerebral ischemia (Zhu et al.

2013). The reason for similar pharmacokinetic profile except brain penetration may be due to the fact piracetam is not extensively metabolized in the liver and is excreted mainly in the unchanged form through urine (Tacconi et al. 1985 and Gouliaev et al. 1994). In a similar observation, pharmacokinetic parameters except for brain penetration of interleukin-1 receptor antagonist are found to be similar (Greenhalgh et al. 2010); however, the pharmacokinetic profile of scutellarin is significantly different during the stroke (Li et al. 2016) indicating that pharmacokinetic profile of a drug may be dependent on the physicochemical property of the drug. Furthermore, the intestinal extraction, portal availability, and hepatic extraction of piracetam after cerebral ischemic-reperfusion injury and control rats were similar; confirm that absorption, distribution, metabolism, and excretion profile of piracetam is not influenced during the pathological condition of cerebral ischemia.

Piracetam is a well-known neuroprotective agent in the management of acute hemispheric stroke (Wustmann et al. 1981; Depresseux et al. 1986 and De et al. 1997) and aphasia (Enderby et al. 1994 and Huber et al. 1999). In the present study, we found that the piracetam ameliorated ischemic brain damage by reducing the infarct volume induced due to focal cerebral ischemic reperfusion injury in the brain. The present study provides evidence of enhanced brain penetration of piracetam after ischemic-reperfusion injury. Our study was designed and performed bearing in mind the complexity that still exists in the establishment of correlation between piracetam plasma and brain levels related in ischemic stroke rats. The present data for the first time compares the pharmacokinetic parameters including brain penetration of the piracetam in control rats and ischemic stroke rats, since it is important to determine the pharmacologically relevant concentration of a drug in pathological condition.

A clear difference in piracetam brain exposure profile was observed between ischemic stroke and control rats. After a single oral dose (200 mg/kg), piracetam was detected in plasma, CSF, and brain, and reduces ischemic brain damage in ischemic-reperfused rats. Our findings suggest that the brain exposure of piracetam increases in the conditions of ischemic stroke and piracetam is readily available to brain tissue in focal ischemia condition. The pharmacokinetic parameters of piracetam obtained from the present study could be applied as a reference for evaluating the clinical efficacy of piracetam.