Improved Brain Energy Metabolism in AβPP-PS1 mouse model of Alzheimer's Disease upon Treatment with Ayurvedic Amalaki Rasayana: A ^{1}H-[^{13}C]-NMR Study

Anant Bahadur Patel[1], Vivek Tiwari[1], Kamal Saba[1], and Subhash C. Lakhotia[2]

[1]*NMR Microimaging and Spectroscopy, CSIR-Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India,* [2]*Department of Zoology, Banaras Hindu University, Varanasi, Uttar Pradesh, India*

INTRODUCTION: Alzheimer's disease (AD) is one of the most common forms of dementia, marked with loss of memory and cognitive functions. AβPP-PS1 mice exhibit severe memory loss and intense plaque load which is the hallmark of the AD[2]. Glucose oxidation and neurotransmitter cycling associated with glutamatergic and GABAergic neurons have been shown to be reduced in AβPP-PS1 mice even at the age of 6 months[3]. Amalaki Rasayana (AR), a traditional Ayurvedic formulation, has been shown to reduce the DNA damage associated with age in neurons and astrocytes[4] and to improve several biological parameters in Drosophila model[5]. The objective of the present study is to evaluate the potential of two drugs AR and Donepezil (Dp) on cognitive function and neuronal metabolism in AβPP-PS1 mice at 12 months of age by using ^{1}H-[^{13}C]-NMR spectroscopy in conjunction with infusion of [1,6-$^{13}C_2$]glucose.

MATERIALS AND METHODS: All animal experiments were performed under approved protocols by the Institutional Animal Ethics Committee. Dp or AR were administered for 30 days to different groups of mice: Group (i) WT+NS, (ii) AβPP-PS1+Normal Saline (NS), (iii) WT+Dp (2 mg/kg), (iv) AβPP-PS1+Dp (2 mg/kg), (v) WT+AR (2 g/kg), (vi) AβPP-PS1+AR (2 g/kg). Learning and memory in AβPP-PS1 mice were assessed using Morris water Maze (MWM) test. Metabolic measurements were performed in overnight fasted mice. Urethane anesthetized mice were administered [1,6-$^{13}C_2$]glucose for 10 min through tail vein using bolus variable infusion rate[6]. Blood was collected and head was frozen *in situ* into liquid nitrogen at the end of infusion. Metabolites were extracted from frozen brain tissues (Cerebral cortex, hippocampus, striatum)[7]. Concentration and percentage ^{13}C enrichment of cerebral amino acids were measured in ^{1}H-[^{13}C]-NMR spectrum (Fig. 2) of tissue extracts acquired at 600 MHz spectrometer[8].

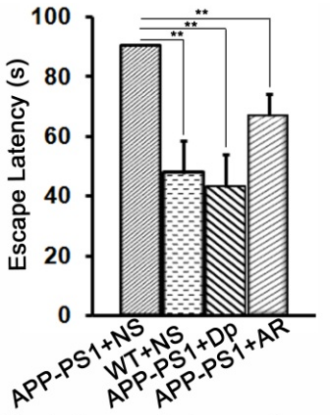

Fig.1: Escape Latency of Mice upon various interventions

RESULTS AND DISCUSSIONS: AβPP-PS1 mice treated with NS could not reach the platform in MWM test suggesting impaired learning and memory. Intervention with AR or Dp improved the learning in AβPP-PS1 mice, and decreased latency to reach the platform (AR: 66±16 and Dp: 42.7±20 s) (Fig. 1). Cortical levels of glutamate (Wild-Type+NS: 13.6±0.2 µmol/g, AβPP-PS1+NS: 12.2±0.1 µmol/g) and NAA (Wild-Type+NS: 7.8±0.1 µmol/g, AβPP-PS1+NS: 7.3±0.1 µmol/g) was found to be significantly lower ($p<0.01$) in AβPP-PS1 treated with NS as compared with age matched control. Administration of AR improved the total level of glutamate (13.0±0.2 µmol/g, p=0.002) and NAA (7.7±0.3 µmol/g, p=0.04) in AβPP-PS1 mice. Furthermore, the reduction in ^{13}C labeling of amino acids in AβPP-PS1 mice was improved upon AR treatment (Fig. 2A). Accumulation of ^{13}C label into cortical Glu_{C4} (AβPP-PS1+NS: 1.18±0.09 µmol/g, AβPP-PS1+AR: 1.51±0.09 µmol/g, p=0.001) and Gln_{C4} (AβPP-PS1+NS: 0.13±0.02 µmol/g, AβPP-PS1+AR: 0.17±0.02 µmol/g, p=0.02) was increased upon AR treatment in AβPP-PS1 mice, suggesting that AR improved the cortical glutamatergic glucose oxidation and total neurotransmission. Similar improvement of energy metabolism and neurotransmission was seen in hippocampal (Fig. 2B) and striatal regions following AR or Dp treatments. The improvement in energy metabolism with AR intervention might be due to reduced DNA damage[4] and enhanced cellular viability. These data suggest that like Donepezil, the traditional Ayurvedic Amalaki Rasayana also has the potential to improve cognitive function and may provide a strategy for the management of AD patients.

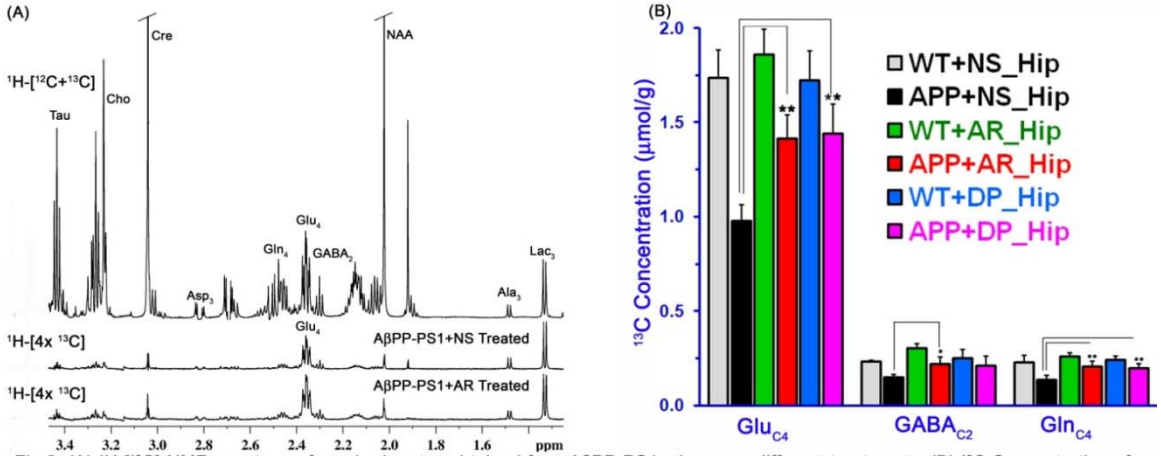

Fig.2: (A) ^{1}H-[^{13}C]-NMR spectrum of cerebral cortex obtained from AβPP-PS1 mice upon different treatments. (B) ^{13}C Concentration of hippocampal amino acids with various interventions, *, $p<0.05$, **, $p<0.01$

REFERENCES: 1. Hardy *et al* (2002) *Science* **297**: 353; **2**. Marjanska *et al* (2005) *Proc Natl Acad Sci* **102**:11906; **3**. Tiwari and Patel (2012) *J Alz Dis* **28**:765; **4**. Swain *et al* (2010) *Mech Ageing Dev* **133**:112; **5**. Dwivedi *et al* (2012) PLoS ONE **7**:e37113**; 6**. Fitzpatrick *et al* (1990) *J Cereb Blood Flow Metab* **10**:170; **7**. Patel *et al* (2001) *Brain Res* **919**:207; **8**. de Graaf *et al* (2003) *Magn Reson Med* **49**:37.

ACKNOWLEDGEMENTS: This study was supported by fundings from Department of Biotechnology (BT/PR14064/Med/30/359/2010) and CSIR network project BSC0208.