Nanoemulsified antioxidant formulations mitigates oxidative stress in chronic sleep fragmented
Human apolipoprotein E4 targeted replacement mice
Ramesh Vijay1, Deepti Nair1, Navita Kaushal1, David Gozal1 and Yashwant Pathak2
1Department of Pediatrics, Pritzker School of Medicine, University of Chicago, Chicago, Illinois
2College of Pharmacy, University of South Florida Health, Tampa, Florida.

Introduction:
Alzheimer’s disease (AD) a progressive brain disorder, pathologically characterized by senile plaques, largely composed of extracellular deposits of β amyloid (Aβ) peptide and neurofibrillary tangles, loss of CBF neurons and attenuation of cortical choline acetyltransferase activity, gradually destroying groups of neurons severely disrupting learning and memory. Relationships between ApoE subtypes (specifically the E4 allele) and AD were first noted in 1993 and have been confirmed many times. ApoE is a polymorphic protein with 299 amino acids and a molecular weight of 34,200 (Mahley, 1988). Among the 3 common isoforms - ApoE2, ApoE3, and ApoE4 - the products of the same gene with three alleles (ε2, ε3, and ε4) at a single gene locus on chromosome 19, ApoE4 is associated as major risk factor for AD. The ApoE3 allele is the most common form typically (70–80%), whereas the ApoE2 and ApoE4 alleles account for only 5–10% and 10–15%, respectively). Protein kinase C is involved in signaling events transduced by ApoE, which is disrupted in AD brain. ApoE3 and ApoE4, Aβ and its complexes, can stimulate the translocation of PK. PKC is known to phosphorylate and inactivate glycogen synthase kinase-3β, a proline directed kinase that has been implicated in the abnormal phosphorylation of AD tau, and known to co-localize with hyper phosphorylated tau in AD degenerating neuron. Therefore, the ApoE isoforms differently induce the expression of genes for signaling intermediates involved in maintenance of cell survival. Patients with AD also experience loss of sleep such as sleep fragmentation (SF). Chronic SF may induce inflammatory responses and oxidative stress. To investigate this, after 15 days of SF, the mice were sacrificed and the brain dissected out for cortex and biochemical estimations for malondialdehyde (MDA) and 8-hydroxy-2-deoxyguanosine (8-OHdG) assays were carried out following 120 days of nano emulsified formulations delivery.

Methods:
The drug delivery studies was conducted in male adult mice (human ApoE4 targeted replacement mice; ApoE4) and Wild type (C57BL/6NTac; WT). Two drugs were administered orally: drug 1, tocopherol (5mg/65 kg body wt), and drug 2, lipoic acid (100mg/65 kg body wt), by gavage, once a day for 120 days. These drugs were administered (1) dissolved in sterile water and (2) nano emulsified form. After the drug treatment, all mice except for the control and vehicle were subjected to SF for 15 days, in a newly designed, developed and validated SF device (Kaushal et al, 2012). Intermittent brief arousals during the entire sleep periods are encountered in various sleep disorders. Chronic SF may induce inflammatory responses and oxidative stress. To investigate this, after 15 days of SF, the mice were sacrificed and the brain dissected out for cortex the lipid peroxidation MDA and 8-OHdG assays were carried out.
Results:
There was a significant decrease in MDA and 8-OHdG following lipoic acid and tocopherol administration. Nano emulsified delivery showed a trend level decrease, although more studies are needed to substantiate the observation. It would be worthwhile to explore more regarding the mechanisms and efficacy of nano emulsified drug delivery. Please see the figure and table.

Discussion:
The nano emulsified drug delivery system enhances bioavailability. Encapsulation of therapeutic agents in nano emulsions can offer improvements in the chemical and/or enzymatic stability of therapeutic agents (Han et al 2009; Nicolaos et al 2003), leading to improvement in shelf-life and/or therapeutic efficacy. Due to the development of the analytical techniques and high tech and ultrasonic homogenizers the globule size can now be reduced to as low as 100 nm and these emulsions are called nano emulsions with globule size from 100 to 500 nm. We have developed and validated a formulation with nano emulsions having globule size 300 to 500 nm. The advantages of such emulsion systems was due to their large surface area, so that release and absorption was enhanced. The fine oil droplets can easily be taken up by the lymphatic systems bypassing the liver metabolism due to its nano size, and can be used to develop drug formulations for various disorders. The development of the nanoparticulate drug delivery systems has opened new vistas in the field of drug therapeutics.

Conclusion:
Studies involving novel drug delivery methods will certainly enhance the chances of better understanding of prevention of oxidative stress-induced progression of AD. Further, use of Nutraceutical nano-emulsions may be developed to deliver drugs to novel therapeutic targets.

References: