SUMMARY AND CONCLUSIONS

ALCOHOL is one of the most commonly used psychoactive drugs in the world. After ingestion, alcohol distributes throughout body tissues and rapidly crosses the blood-brain barrier. It is not surprising that ethanol abuse significantly contributes to damage in variety of tissues including the peripheral and central nervous systems. Alcoholic peripheral neuropathy is a serious and frequently observed consequence of chronic alcoholism (McLane, 1987). It is a potentially incapacitating complication of chronic consumption of ethanol, characterized by pain and dysesthesias, primarily in the lower extremities and is poorly relieved by available therapies (Ratcliff, 1979; Monforte et al., 1995; Ortiz-Plata et al., 1998). Alcoholic neuropathy was thought to be a disorder involving decreased nerve function caused by damage that results from the long-term consumption of excessive alcohol and is characterized by spontaneous burning pain, hyperalgesia and allodynia (Ortiz-Plata et al., 1998). The etiology of alcoholic peripheral neuropathy is characterized by a number of interwoven pathways including activation of spinal cord microglia, oxidative stress leading to free radical damage to nerves and release of pro-inflammatory cytokines coupled with activation of PKC (Dina et al., 2000; Narita et al., 2007).

Alcohol-induced brain damage produces some of the most insidious effects of alcoholism, including cognitive deficits such as learning and memory impairment (Pfefferbaum et al., 1998; White, 2003). Alcohol dependence affects over 14% of the United States population and is the second leading cause of dementia in USA (Kessler et al., 1994). Heavy prenatal alcohol exposure is associated with widespread neuropsychological deficits across several domains including general intelligence, memory, language, attention, learning, visuospatial abilities, executive function, motor skills and social and adaptive function (Mattson and Riley, 1998; Mukherjee et al., 2006). Children exposed to alcohol prenatally also have decreased academic achievement and higher rates of learning disabilities than non-exposed children which may relate to impairments in verbal and non-verbal learning and memory (Roebuck-Spencer and Mattson, 2004). The cost of caring for
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children with FASDs has been estimated at approximately US$ 74.6 million per year, with three quarters of this cost associated with the care of FASD cases with mental retardation (Abel and Sokol, 1991). Therefore, understanding how prenatal alcohol exposure produces behavioral and cognitive deficits is of great medical and economic importance.

With this background, the present study was designed to evaluate the impact of tocotrienol, epigallocatechin gallate, resveratrol and curcumin on alcoholic peripheral neuropathy and alcohol-induced cognitive deficits in both adult and neonatal rats. Moreover, the involvement of oxidative-nitrosative stress and inflammatory cascade in the development of these complications was elucidated.

Chapter 1: Suppression of Oxidative-Nitrosative Stress Mediated Neuroinflammatory Cascade in Experimental Model of Alcoholic Neuropathy

Alcoholic neuropathy represents an important complication of chronic alcohol consumption involving spectrum of structural, functional and biochemical alterations in peripheral nerves. The present study was designed to explore the effect of tocotrienol (50, 100 and 200 mg/kg), epigallocatechin gallate (25, 50 and 100 mg/kg), resveratrol (5, 10 and 20 mg/kg) and curcumin (15, 30 and 60 mg/kg) on chronic alcohol-induced peripheral neuropathy. Chronic alcohol administered rats exhibited marked allodynia and hyperalgesia (thermal and mechanical) at 6th week of alcohol administration. The enhanced pain sensitivity was accompanied by marked increase in oxidative-nitrosative stress in sciatic nerve and cytokines (TNF-α, IL-1β and TGF- β1) release both in serum and sciatic nerve of alcohol administered rats. Co-administration of tocotrienol (50, 100 and 200 mg/kg), epigallocatechin gallate (25, 50 and 100 mg/kg), resveratrol (5, 10 and 20 mg/kg) and curcumin (15, 30 and 60 mg/kg) significantly and dose-dependently prevented various behavioral, biochemical and molecular alterations associated with chronic alcohol administration in rats.
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Therefore, the major finding of the study is that apart from antioxidant activity, the suppression of nitrosative stress and elevated cytokine (TNF-α, IL-1β, TGF-β1) levels by tocotrienol, epigallocatechin gallate, resveratrol and curcumin both in serum and in sciatic nerve of rats also contribute significantly in preventing alcoholic neuropathy.

Chapter 2: Amelioration of Alcohol-Induced Cognitive Deficits by Blocking Inflammatory Signaling and Cell Death Cascade in both Adult and Neonatal Rat Brain

Alcohol-induced cognitive deficit is one of the hallmarks of neuroinflammation which results from chronic and excessive consumption of alcohol. Chronic alcohol intake is known to induce the selective neuronal damage associated with increase oxidative-nitrosative stress and activation of inflammatory cascade finally resulting in neuronal apoptosis and dementia. This study was designed to investigate the effect of tocotrienol, epigallocatechin gallate, resveratrol and curcumin against chronic alcohol-induced cognitive deficits and neuroinflammatory cascade leading to neuronal apoptosis in both adult and neonatal rat brain. Morris water maze and elevated plus maze was used for behavioral assessment of memory. Cytoplasmic and nuclear fractions of cerebral cortex and hippocampus were prepared for the quantification of acetylcholinesterase activity, oxidative-nitrosative stress, TNF-α, IL-1β, NF-κβ and caspase-3. After 6 weeks of ethanol administration in adult rats and postnatal ethanol exposure in rat pups, produced significant memory impairment in both the behavioral paradigms of memory assessment which was coupled with enhanced acetylcholinesterase activity, increased oxidative-nitrosative stress, TNF-α, IL-1β, active p65 subunit of NF-κβ and caspase-3 activity in different brain regions of ethanol treated animals. Interestingly, co-administration of tocotrienol (50, 100 and 200 mg/kg), epigallocatechin gallate (25, 50 and 100 mg/kg), resveratrol (5, 10 and 20 mg/kg) and curcumin (15, 30 and 60 mg/kg) significantly and dose-dependently prevented behavioral, biochemical and molecular changes in ethanol administered adult rats. Moreover, tocotrienol (50 and 100 mg/kg), epigallocatechin gallate (50 and 100 mg/kg), resveratrol
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(10 and 20 mg/kg) and curcumin (30 and 60 mg/kg) treatment also produced significant amelioration in cognitive deficits along with attenuation of associated biochemical and molecular alterations in different brain regions of ethanol exposed neonatal rats.

Collectively, the data reveals that oxidative-nitrosative stress mediated inflammatory cascade resulted in activation of apoptotic signaling pathway and may contribute to the learning and memory deficits in ethanol exposed animals. Treatment with tocotrienol, epigallocatechin gallate, resveratrol and curcumin prevents cognitive deficits by blocking oxidative-nitrosative stress mediated activation of apoptotic signaling pathway and thus has a potential to be a useful therapeutic option against patients with alcoholic dementia and in children with fetal alcohol spectrum disorders.

Chapter 3: Mechanistic insight in the pathophysiology and amelioration in experimental models of alcoholic neuropathy and encephalopathy

Pearson correlatin analysis showed that curcumin produced more beneficial effects in ameliorating alcoholic neuropathy while epigallocatechin gallate found to be more potent in preventing chronic alcohol-induced cognitive deficits in rats. To gain deeper insight in to the mechanistic pathophysiology involved in chronic alcohol-induced neuropathy and associated cognitive deficits and their amelioration by curcumin and epigallocatechin gallate respectively, the involvement of substance P (SP) and nerve growth factor (NGF), important mediators of neuropathic pain, in alcoholic neuropathy while brain derived neurotropic factor (BDNF), marker of neurogenesis, in alcohol-induced cognitive deficits was studied. The study was divided into two subchapters.

Chapter 3.1: Curcumin prevents alcoholic neuropathy in rats by attenuating substance P and nerve growth factor

Substance P and nerve growth factor are known to play a very important role in pain and nociception specifically inflammatory pain. As alcoholic neuropathy also involves increase in inflammatory mediators, the
present study was designed to explore the role of SP and NGF in alcoholic neuropathy and the effect of curcumin administration on these parameters. Chronic alcohol administration for 10 weeks resulted in significantly decreased nociceptive threshold along with increased oxido-nitrosative stress, pro-inflammatory cytokines (TNF-α, IL-1β, TGF-β1), substance P and nerve growth factor levels both in serum and sciatic nerve of rats and treatment with curcumin significantly prevented all the behavioral, biochemical and molecular deficits. Thus, attenuation of oxidative-nitrosative stress, elevated cytokine (TNF-α, IL-1β, TGF-β1), substance P and NGF levels both in serum and in sciatic nerve by curcumin contribute significantly in preventing alcoholic neuropathy in rats.

Therefore, the major finding of the study is that curcumin treatment significantly prevented thermal and mechanical hyperalgesia, and tactile allodynia in rats chronically fed with ethanol by modulating neuroinflammatory signaling and attenuating substance P and NGF levels and thus curcumin may find a place in therapeutic armamentarium for the treatment of patients suffering from alcoholic neuropathy.

Chapter 3.2: Epigallocatechin gallate prevents cognitive deficits in rats by attenuating alcohol-induced inhibition of neurogenesis

The neurotoxic effects of alcohol have been confirmed both in human and animal studies, providing evidence of the vulnerability of the brain to the effects of ethanol and the long-term cognitive consequences (learning and memory deficits) of chronic alcohol administration. Apart from alcohol-induced neuroinflammation and resulting cell death, alcohol-induced inhibition of neurogenesis has also been proposed as a possible mechanism of chronic alcohol-induced cognitive deficits. This study was designed to investigate the effect of epigallocatechin gallate and memantine against chronic alcohol-induced cognitive deficits, neuroinflammatory signaling cascade and brain derived neurotropic factor (marker of neurogenesis). Morris water maze and elevated plus maze was used for behavioral assessment of memory. Cytoplasmic and nuclear fractions of cerebral cortex and hippocampus were
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prepared for the quantification of acetylcholinesterase activity, oxidative-nitrosative stress, TNF-α, IL-1β, NF-κβ, caspase-3 and BDNF levels. After 6 weeks of ethanol administration, rats exhibited significant memory impairment in both the behavioral paradigms of memory assessment which was coupled with enhanced acetylcholinesterase activity, increased oxidative-nitrosative stress, TNF-α, IL-1β, NFκβ and caspase-3 activity in different brain regions of ethanol treated animals. Apart from this, BDNF levels were also significantly decreased in both the brain regions of ethanol administered rats suggesting inhibition of neurogenesis. Epigallocatechin gallate and memantine treatment significantly ameliorated behavioral, biochemical and molecular changes in ethanol administered rats. Taken together, the emerging consensus is that ROS, RNS, TNF-α and IL-1β are likely to be the general modulators in NF-κβ signaling pathway and caspase-3 activation and may contribute to the learning and memory deficits in rats chronically exposed to ethanol. Apart from this decreased BDNF levels also play an important role in potentiating neurotoxic effects of alcohol and mediating cognitive deficits in rats chronically administered ethanol.

The results of the present study revealed that treatment with epigallocatechin gallate significantly prevented cognitive deficits induced by chronic ethanol administration not only by attenuating apoptotic signaling but also by preventing ethanol-induced inhibition of neurogenesis in different brain regions of rats.

Thus, Curcumin and EGCG have a potential to be developed as therapeutic options for patients with alcoholic neuropathy and alcoholic dementia respectively.