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Alcohol aggravates stress-induced cognitive deficits and hippocampal neurotoxicity: Protective effect of melatonin

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ABSTRACT

Stressful events and alcohol abuse are the cumbersome situations which can synergistically predispose the negative effects on the brain. Oxidative stress generated by chronic immobilization and alcohol consumption cause severe neurotoxicity in the hippocampus region that ultimately leads to cognitive dysfunction. In the current study, we have investigated the involvement of NF-κB/Nrf/HO-1 transduction pathway in stress and alcohol exposed animals. Male Swiss albino mice were given alcohol (ALC) (15% v/v) or restraint stress (RS) or both (RS for 6 h per day) up to 28 days. We found increased ALC consumption in the ALC + RS group as compared to the ALC group. Morris water maze (MWM) test and novel object recognition test (NORT) revealed the spatial and recognition memory impairment in RS and ALC + RS group. ALC + RS group showed more profound oxidative stress and augmentation of pro-inflammatory cytokine (IL-1β) as compared to RS or ALC group alone. Melatonin (20 mg/kg, p.o) treatment for 14 days significantly prevented the raised oxidative stress, release of IL-1β, GSH depletion and augmentation of AChE activity in the hippocampus. Moreover, semi-quantitative reverse transcriptase PCR results showed that combined exposure of ALC and RS leads to over-activation of NF-κB transduction inflammatory pathway and down-regulation of the Nrf2/HO-1 axis which was significantly ameliorated by the melatonin treatment. In conclusion, our results indicated that ALC + RS exerted the deleterious effects on the hippocampus which were alleviated by the melatonin treatment.

1. Introduction

The modern lifestyle, environment, and dietary factors have always been considered primarily in raising the risk of various health issues such as neurological, cardiovascular diseases and cancers etc. Hippocampus structure inside the brain is a sensitive part that generally involved in the learning and memory behavior which remains vulnerable to life-long stresses. Numerous reports suggested that external stress generates oxidative/nitrosative stress through activation of several inducible enzymes (such as inducible nitric oxide synthase (iNOS), Cyclooxygenase-2 (COX-2), release of pro-inflammatory cytokines (such as interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-α)) which leads to inflammation in different brain regions [1–3]. Repetitive active stress provokes the over-activation of hypothalamic-pituitary-adrenal (HPA) and sympathoadrenomedullary (SAM) axis that further turn on the downstream cascade. This further leads to activation of adrenal gland that releases the cortisol and catecholamines for combating the stressful response [4]. The chronic stress leads to hippocampus atrophy by inhibiting neurogenesis and inducing apoptotic neuronal death that eventually promotes cognitive dysfunction and neuropsychiatric illness [5,6].

Alcohol (ALC) abuse is the key problems of destructing socioeconomic burdens as well as normal health status. World Health Organization (WHO) reported 3.3 million (5.9%) deaths every year due to heavy alcohol consumption [7]. ALC drinking relieves stress in the initial stage but later on hold the debilitating impact on physical as well as psychological health. Chronic consumption of alcohol may lead to various aversive events such as memory impairment, anxiety disorders, depression, renal and liver failure and teratogenic effects [8–11]. Moreover, the acute and chronic ALC drinking induces several epigenetics changes which eventually modulate certain gene expression in various brain

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regions [12,13]. The clinical studies revealed the shrinkage of hippocampus and cognitive decline with ALC consumption [14].

The stress-alcohol interactions are of multifarious nature and difficult to understand. Earlier reports provide the mixed reviews for the animal with the variety of drinking patterns (influence drinking, reduction, and no change) under different stressful conditions [15]. Moreover, several factors such as age and corticotrophin-releasing factor are involved in modulating the ALC drinking mediated by acute/chronic stress [16–18]. Both stress exposure and ALC consumption causes behavioral and neurochemical anomalies in the hippocampus [19]. The RS and ALC are able to generate oxido-nitrosative stress and pro-inflammatory cytokines release thereby activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway in different brain regions including hippocampus. This occurs through activation of neuron cell bodies and glial cells in the brain [20,21]. It may further lead to the alteration in hippocampal volume, redox balance, morphology, and function [22,23].

The antioxidants play a vital role in protecting the brain from oxidative stress and neuronal insult by neurotoxicants [3,5,24,25]. Melatonin (N-[2-(5-Methoxy-1H-indol-3-yl) ethyl] acetamide) is an endogenous potent antioxidant, secreted hormone from pineal gland with its important role in regulating the sleep cycle. Previous reports suggested numerous neuroprotective activities of melatonin in various models such as rodent model of diabetes, ischemia, and ethanol-induced reactive gliosis [26–28]. A previous study demonstrated that melatonin exerts neuroprotective effect against ethanol-induced memory deficits [29]. Moreover, another study showed the neuroprotective effect of melatonin against brain injury via activation of the Nrf2/ARE pathway [30]. Therefore, the current study was designed to evaluate the combined effect of restraint stress and alcohol exposure on neurobehavioural and neurochemical alterations. In addition, we have evaluated the protective effect of melatonin against restraint stress and alcohol-induced neuronal insult.

2. Material and methods

2.1. Chemicals

Melatonin and Interleukin-1β (IL-1β) were purchased from TCI Chemicals (India) Pvt. Ltd and Thermo Fischer scientific, India respectively. Ethanol was procured from SD fine chem. Limited (Taratala, Kolkata, India). Total RNA extraction kit (Hi-Media India), Verso cDNA Synthesis Kit (Thermo Fisher Scientific India), and primers (Imperial life sciences (P) Limited India) were purchased. All other chemicals used in the study were of analytical grade and purchased from sigma Aldrich (St. Louis, MO, USA).

2.2. Animals

Adult male Swiss albino mice (weight: 25–30 g) of age 8 weeks old were procured from the Central animal facility of the Institute (Gauhati Medical College, Guwahati). The experimental protocol was approved (approval NO. MC/05/2015/21) by the Institutional Animal Ethics Committee (IAEC), Guwahati Medical College & Hospital (CPSCEA Registration No. 351:3/1/2001). The animals were kept in standard environmental conditions (24 ± 1 °C; humidity 65 ± 5%; reversed 12 h light/dark cycle). Mice were housed in commercially available cages (n = 6 per cage) and provided food and water ad libitum. The animals were acclimatized for the 14 days prior to the commencement of the experiment. All the experimental procedures were conducted between 9:00 and 15:00 h and were carried out in accordance with the guidelines of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India.

2.3. Experimental design and treatment schedule

Initially, mice were trained to prefer ALC through the two-bottle choice paradigm. Ethanol concentration was gradually increased from 1 to 15% v/v during 15 days of the training phase. Afterward, seven experimental groups were taken each containing 12 mice. Fig. 1 shows the study timeline of experimental study.

Group1 as the control group: normal saline (0.9%) was administered for last 14 days. Group 2 as Alcohol (ALC) drinking group: 15% v/v alcohol given to trained mice for 28 days. Group 3 as Restraint stress (RS) group: Animals were restrained 6 h/day for 28 days. Group 4 as Alcohol (ALC) + Restraint stress (RS) group: Trained animals were given alcohol (15% v/v) and restrained 6 h/day for 28 days. Group 5: Melatonin (20 mg/kg) was administered orally from 15th to 28th day and alcohol (15% v/v) was given for 28 days. Group 6: Melatonin (20 mg/kg) was administered orally from 15th to 28th day and restrained 6 h/day for 28 days. Group 7: Animals were restrained 6 h/day and given alcohol (15% v/v) for 28 days. In addition, group 7 was treated with melatonin (20 mg/kg) from 15th to 28th day. Alcohol and water bottles were removed from non-restrained groups during restrained stress procedure. Group 2, 3 and 4 were treated with 0.9% saline from 15th to 28th day. Alcohol consumption in different groups was measured on the

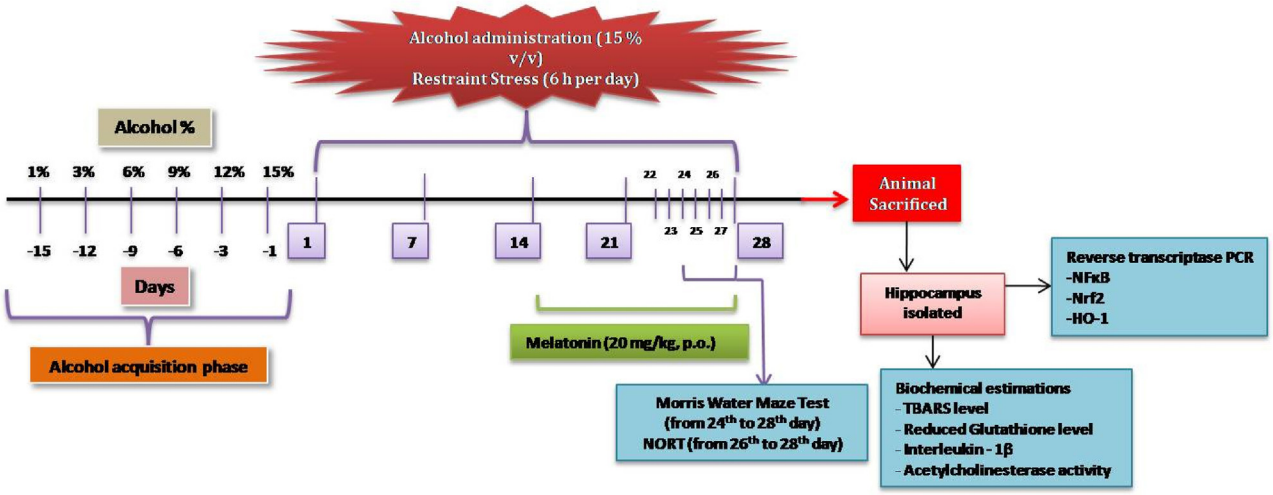

Fig. 1. Illustration of timeline study plan.