Protective Effect of *Ocimum sanctum* on Ethanol-induced Oxidative Stress in Swiss Albino Mice Brain

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Abstract:

Chronic ethanol consumption is a medical problem with important socio-economic repercussions worldwide. Its chronic consumption enhances the oxidative damage to neurons and resulting in cell death. In this study we evaluated the protective effect of *Ocimum sanctum* on ethanol-induced oxidative stress in swiss albino mice brain. Mice were divided into groups comprising of vehicle control, ethanol control, *Ocimum sanctum* water extract (OSWE) treatment at three doses, quercetin as a reference drug control and the treatment was given orally for 30 days. Oxidative stress and anti-oxidant related enzymes were estimated from brain homogenate. OSWE was found to inhibit the lipid peroxidation and nitric oxide and enhance the superoxide dismutase in dose dependent manner.

Key words: Ethanol, *Ocimum sanctum*, Ethanol Oxidative Stress, Mice.

Introduction

Ethanol is the most psychoactive substance and chronic ethanol consumption is a medical problem with important socio-economic repercussions worldwide (Sandhir and Gill, 1999). Besides liver damage, chronic alcohol consumption is associated with several degenerative and inflammatory processes in the central nervous system (CNS) includes enhance reactive oxygen species(ROS) production in brain through a number of pathways including increased generation of hydroxyethyl radicals(Perez-Campo *et al.* 1998; Rezvani *et al.,* 2003), induction of CYP2E1, alteration of the cytokine signaling pathways for induction of inducible nitric oxide synthase and, phospholipase A_2 and production of prostanoids through the cyclo-oxygenase pathways.(Suna and Snub,2001). The brain is deficient in oxidative defense mechanisms and hence is at great risk of damage mediated by ROS resulting in molecular and cellular dysfunction. Therefore, it is possible that oxidative changes exerted by chronic and excessive ethanol consumption may exacerbate the progression of neurodegenerative disorders (Kumral *et al.,* 2005).It is widely accepted that alcoholism is a complex heterogeneous disorder that involves multiple mechanisms that can damage the brain. Various finding indicate that chronic ethanol consumption leads to direct or indirect changes in the viability of central nervous system cells via oxidative stress.

Ocimum sanctum (family Labiatae), the Indian holy basil, commonly known as sacred Tulsi, is a fragrant bushy plant found in semi tropical and tropical parts of India. Different parts of the plant are traditionally used in the Ayurveda and Siddha systems of

medicine for treating infections, skin diseases, hepatic disorders, cold, cough, malarial fever and as an antidote for snake bite (Satyvathi and Gupta, 1987). Various studies on *Ocimum sanctum* have shown protection against radiation induced lipid peroxidation with increased levels of cellular antioxidants (Uma Devi and Ganasoundari, 1999),immunotherapeutic potential in bovine sub-clinical mastitis (Mukherjee *et al.*, 2005), cardioprotective effect (Sood *et al.*, 2006) and also inhibition of noise induced neurotransmitter levels (Samson *et al.*, 2006). Medicinal plants have been in use for the treatment of ethanol dependency have only recently attracted the attention of scientists. Therefore, a more extensive investigation was undertaken to understand effect of *ocimum santum* on ethanol induced oxidative stress in swiss albino mice brain.

Materials and Methods

Plant Material

Fresh Leaves of *Ocimum sanctum* were collected early in the morning from CIMAP research field, Lucknow-India and voucher specimen of plant material was deposited at the institute's herbarium. The leaves were washed, dried in the shade and ground to a fine powder (# 60 mesh) using a laboratory mixer. One hundred grams of leaf powder was refluxed with 750ml of double distilled water for one hour at 40^0C and concentrated using rotavapour (Buchi India). The extract was kept in -20^0C until used for experiment.

Experimental Design;

Inbred swiss albino female mice weighing 18-25 gm obtained from 'Jeevanika', (Animal house of CIMAP), Lucknow were used for the ethanol induced oxidative stress study. The animals were maintained at 22±3°C with 50-70% relative humidity and 12:12

hrs of light and dark cycles. The animals were fed with pellet diet procured from M/S Dayal industries, Lucknow, India, containing 22-24% protein ,4-5%fat, 4-5%crude fiber, nitrogen free extracts 45-55%, bengal gram 15%,phosphorus 0.4-0.6%, calcium 1-1.5%, insoluble ash 8% and soaked bengal grams and water *ad libitum*. Animals were divided into groups (n=6) as vehicle control(0.7% CMC), ethanol control(18%v/v) @ 5gm/kg/day), OSWE(10,30 and 100mg/kg body weight) and quercetin (75mg/kg body weight) as a plant derived standard antioxidant, all of which received an identical volume (0.2ml/mice) of treatment and ethanol by oral rout of administration using oral feeding needle. Preventive effect of OSWE against the ethanol-induced oxidative stress in mice was followed as per the method described by (Molina *et al.*, 2003). Experimental mice were pre-treated with OSWE and quercetin for 15 days prior the chronic administration of the ethanol. Administration of test compound was continued along with the ethanol. On 3rd week of ethanol administration, treatment of the test compound was stopped and administration of ethanol was continued for another 15 days (Figure No.1). All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

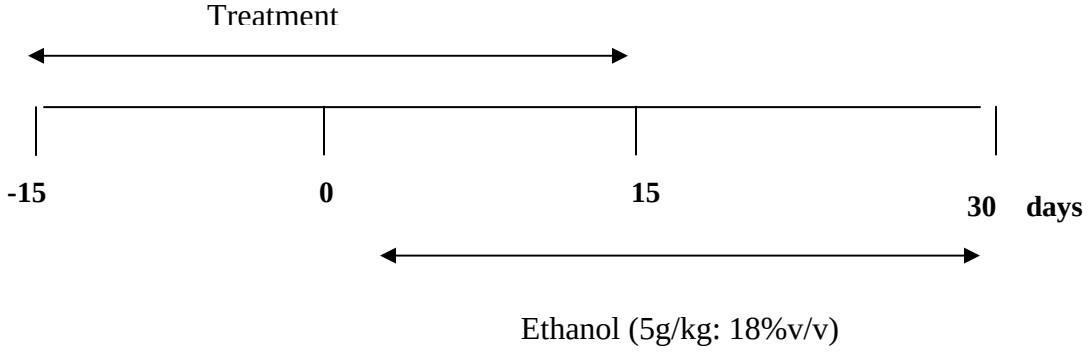

Figure No.1:Pre-treatment scheduled of oral administration of OSWE in ethanol induced oxidative stress model in mice

Preparation of tissue homogenate:

Twenty four hour after the last administration, all the animals were anaesthetized and sacrificed by cervical dislocation. The brain was dissected out from cranial cavity and was immediately placed in a beaker containing ice-cold PBS (pH 7.4) which was minced into small pieces and homogenized immediately in polytron homogenizer (Pro Scientific Inc, Monroe,CT, USA) under the cold condition and kept it in -20^{0}C till the further processing for the estimation of, malondialdehyde (MDA) as a marker of lipid peroxidation, nitric oxide (NO) and superoxide dismutase (SOD) enzyme activity.

Estimation of lipid peroxidation

The quantitative measurement of lipid peroxidation was performed following the standard thiobarbituric acid (TBA) assay. The amount of MDA formed was quantitated by reaction with TBA and used as an index of lipid peroxidation. The results were expressed as MDA (µM/g wet tissue).

Estimation of Nitric Oxide (NO) production

Nitric Oxide production was estimated as nitrite released from tissue homogenate. To measures the nitrite content, 100µl tissue homogenate was mixed with equal volume of griess reagent (Sigma Aldrich, USA) and incubated at room temperature for 10 minutes. The absorption at 540 nm was determined in a microplate reader. Nitric oxide estimation was carried out using standard curve plotted against the known quantity of sodium nitrite ($NaNO_2$) .The results were expressed as µmol nitrite/ml tissue homogenate as per the method described by Freitas *et al.*,2005.

Estimation of super oxide dismutase (SOD) an antioxidant enzymes:

SOD was estimated as per the method described by Madesh and Balasubramanian (1998). It involves generation of superoxide by pyrogallol autoxidation and the inhibition of superoxide-dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiazol 2-xl) 2,5 diphenyl tetrazolium bromide] to its formazan, measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazan formed. The colour evolved is stable for many hours and is expressed as SOD Units /mg of tissue homogenate.

Results and Discussion

Medicinal plants are recognized for their ability to produce a wealth of secondary metabolites and its used in disease treatment has been in practice from several generations. Our understanding of the scientific principles of these herbal drugs is still unsatisfactory, resulting in the limitation of their widespread use in patients. The main objective of the present study was to evaluate the protective effect of *Ocimum sanctum* on ethanol-induced oxidative stress on mice brain. Chronic exposure of ethanol caused significant alteration in oxidative stress-related indices in brain homogenate. Malonaldehyde (MDA), a marker of lipid peroxidation(LPO) and nitrite, a marker of nitric oxide (NO) formation was significantly increased and superoxide dismutase (SOD), an anti-oxidant enzyme was significantly inhibited in ethanol treated group when compared with vehicle control group($P<0.05$). Due to the presence of high proportions of polyunsaturated fatty acids and low oxidant defense enzymes in the brain, this organ is particularly susceptible to oxidative stress, and free radicals are generated under normal

as well as pathological conditions [Gilman *et al.*,1993:Skaper *et al.*,1999]. Ethanol metabolism leads to the production of highly reactive molecules such as indirect metabolites malondialdehyde, 4-hydroxynonenal (Montoliu *et al*., 1995), superoxide and hydroxyl radicals generated during the metabolism of ethanol by the microsomal oxidising system that can destroy vital cell components through a process called oxidation which may be involved in the pathogenesis of alcohol-related tissue injury(Fernandez-Checha *et al.,* 1996; Rouach *et al.,* 1997). Chronic ethanol administration induces oxidative stress in the central nervous system, mainly through increased lipid peroxidation of the cell membrane leading to increased membrane fluidity, disturbances of calcium homeostasis (increase in free intracellular calcium) and finally cell death (Hansson *et al.*,1990; Montoliu *et al.*,1995). The inducible form of NOS (iNOS, type II) produces a much higher amount of NO and is an important mediator of the inflammatory reaction in the human body. Diffusion of NO into pathogenic cells leads to inactivation of some enzymes and the formation of reactive oxygen species (Galea *et al.,* 1992; Murphy *et al.,* 1998).

LPO and NO level were significantly reduced ($P<0.05$) and SOD level was enhanced in OSWE treated groups in a dose dependent manner when compared with the ethanol control group. The respective data is depicted in figure no.1, 2 and 3. Several previous reports have demonstrated that *ocimum sanctum* exhibiting antioxidant and neuroprotective effect on transient cerebral ischemia and long-term cerebral hypoperfusion (Yanpallewar,2004) and nitric oxide scavenging activity (Jagetia and Baliga 2004), cardiac endogenous antioxidants and prevents isoproterenol-induced myocardial necrosis in rats, inhibition of lipid peroxidation: in vivo and in vitro

studies(Geetha and Vasudevan, 2004), radioprotective properties(Subramanian et al., 2005), peptic ulcer model(Kath and Gupta,2006), would healing model(shetty et al., 2006) on cardiac changes in rats subjected to chronic restraint stress(Sood et al., 2006) the anti-oxidant activity in noise exposure model (Samson et al., 2007).

The result of the present study concluded that the *Ocmium sanctum* water extract can protect the brain damage due to the chronic consumption of ethanol can be used as prevention therapy in chronic ethanol consumption people to avoid the damage of the vital organs.

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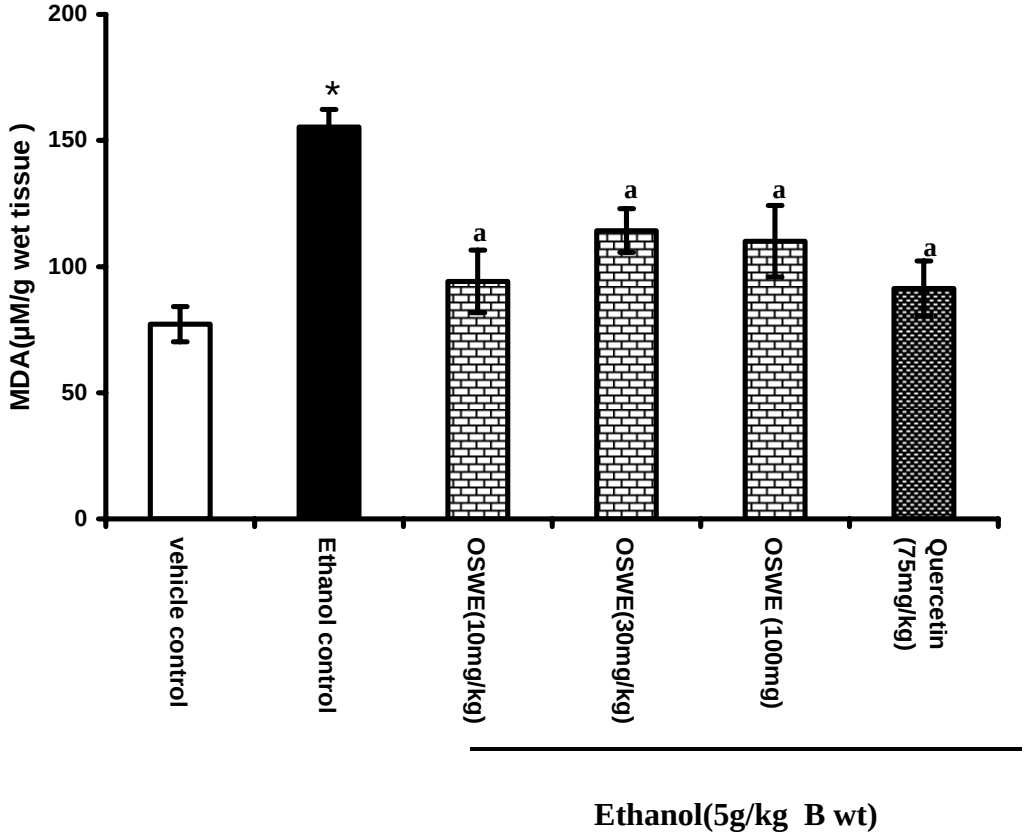
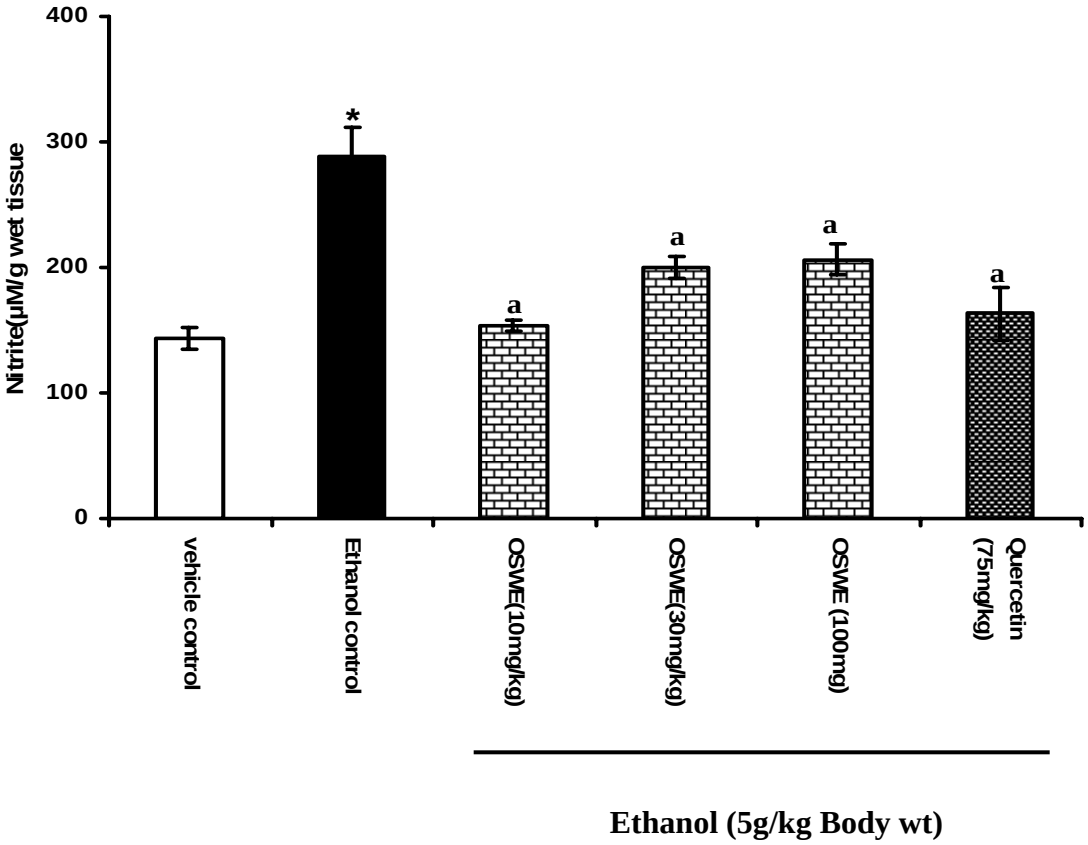
Figure No.1: Effect of OSWE on MDA formation in ethanol-induced oxidative stress mice brain.



n=06, P<0.05,*Vehicle control verses Ethanol control

[a] Ethanol control verses Ethanol +Treatment

Figure No.2: Effect of OSWE on Nitrite formation in ethanol-induced oxidative stress mice brain.



n=06, P<0.05,*Vehicle control verses Ethanol control

[a] Ethanol control verses Ethanol +Treatment

Figure No.3: Effect of OSWE on SOD formation in ethanol-induced oxidative stress mice brain.