Original Article

Extremely Dilution of *Strychnos Nux vomica* Mitigates Alcohol-Induced Reduction in Enthalpies Associated With Free Water Molecules in a Fish Brain

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Abstract

Introduction: Alcohol intoxication affects aquaporins in the glial cells of brain resulting in oedema. *Nux vomica*, a homeopathic drug of plant origin, is known to counteract alcohol effect. The objective of this present study is to find out the level of free water molecules in the brain of a teleost fish under ethanol intoxication. The second objective is to determine whether *Nux vomica* could restore the level of free water in the alcohol treated fish. **Materials and methods:** One group of fish was exposed to 456 mM ethanol for 30 min, another exposed first to a solution of *Nux vomica* 200c for 20 min and then to 456 mM ethanol for 30 min. The third group served as an untreated control. The mid brain of each fish was kept in an aluminium sample pan and its free water level was assessed by differential scanning calorimetry (DSC). **Results**: All alcohol treated fish showed significant reduction in the level of free water molecules as compared to the untreated control. Treatment with *Nux vomica* increased the level of free water in the brain significantly as compared to the untreated alcoholic group. **Conclusion:** Alcohol intoxication reduces free water molecules in the fish brain. *Nux vom* might have acted on aquaporins in the glial cells thereby increasing the level of free water in the brain.

Key words: Free water molecules, Aquaporin 4, *Nux vomica*, Alcohol, Brain.

Introduction

Alcoholism is a global health problem. Alcohol consumption results in loss of memory, high blood pressure, weakness of muscles, hepatic disorders, low immune function, cardiac problems, anaemia, pancreatic disorders and mental depression (Wikipedia, 2016). Extract of the seeds of *Strychnos Nux vomica* and its high dilution have long been used in homeopathy for alcohol induced diseases of patients[1,2]. High dilution of *Nux vomica*

significantly reduces alcohol induced sleep time in albino mice[3]. *Nux vomica* extract and its high dilution reduce voluntary ethanol intake in rats[4]. Results of animal studies on alcohol toxicity bear relationship with findings in humans[5]. Alcoholism leads to reduced brain volume[6]. Glial cells like astroglia contain large number of water channel proteins or aquaporin (AQP4) which mediate glial oedema resulting from ethanol intoxication[7]. However, it is not known whether alcohol could affect the level of free water molecules in the brain. The purpose of the present study is to see whether ethanol would alter free water content in the brain of a teleost fish (*Channa punctatus*). The second objective is to see whether high dilution of *Nux vomica* could counter the alcohol-induced change in the level of free water in a fish brain. In a series of experiments we have demonstrated that drugs at ultra high dilution differ from each other with respect to free water molecules and hydrogen bond strength[8-11]. Free water molecules play an important role in the development of cataract in human lenses[12].

Materials and Methods

Live fishes (*Channa punctatus*) weighing 19-22g were collected from fish market and kept in an aquarium at a temperature $22^0C \pm 2^0C$ in the laboratory with constant aeration of water by bubbling. Water in the aquarium was changed once in 24 hours. The fishes were exposed to natural light and dark cycle. After 6 days fishes were taken from the aquarium for experimental studies. *Channa punctatus* lives in small fresh water ponds and ditches where water level comes down in summer. The fish is able to live in shallows during long drought in summer times.

Three groups of fishes, each comprising 5 individuals, were kept in round glass fish bowls just before treatment.

Group 1: Without treatment (Control)

Group 2: Treated by immersion in anaesthetic dose of EtOH (456 mM EtOH) for 30 min

Group 3: Pre-treated by immersion with *Nux vom* 200c in 90% ethanol diluted with distilled water 1:100 for 20 min followed by treatment with anaesthetic dose of EtOH (456 mM EtOH) for 30 min

All concentrations of alcohol were checked in a UV-Vis spectrophotometer at 200 nm and found to be same, thereby confirming their ethanol content. For treatment with *Nux vom* 200c fishes were immersed in 250 ml of *Nux vom* 200c solution for 20 minutes in a glass bowl. After treatment all the fishes were taken out, kept in a nylon net and washed with tap

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water for 2 min. For treatment with alcoholic solution fishes were immersed in ethanol solution (456 mM EtOH) for 30 min and then washed with water in a similar way. Immediately after the treatment each fish was first decapitated and its brain was taken out and placed on a glass slide. A small part of the mid brain (15.5 mg) was cut out and put in an aluminium sample pan of 5 mm diameter, and sealed by a sealing machine.

DSC measurement

DSC measurements were carried out by using 200 F3 Maia model instrument with intra cooler 70 version (NETZSCH), Germany. The experiment was conducted in dry nitrogen atmosphere with constant pressure of 0.3 bar in order to prevent any oxidation of the samples. A vacant pan was measured first as a reference.

DSC of each sample was measured from a starting temperature of 28^0C down to -35^0C at a scanning rate of 5K min^{-1} and kept at -35^0C in an isothermal condition for 5 minutes. Then the temperature was raised at 3K min^{-1} upto 50^0C[12]. In this way freezing and melting points of each sample was recorded and exothermic (freezing) and endothermic (melting) enthalpies were calculated. Mass of freezable water or free water is directly related to its melting enthalpy[11,13]. Usually, freezable water increases linearly with the increase in water content in a hydrophilic polymer[13].

Results

DSC curves of a representative sample from each group of fish is presented in figures 1 and 2 showing freezing and melting points, respectively. Figure 1 shows some minute variations in freezing points of brain samples. However, variation in melting points is negligible (Fig. 2). All data concerning the melting and freezing enthalpies are presented in histogram in figure 3. The results show that there was no significant variation in melting and freezing temperature of brain samples but the enthalpies, both freezing and melting varied between *Nux vom* treated group and the untreated groups (Figure 3). Alcohol treatment leads to a significant reduction of enthalpies vis-a-vis free water molecules in the mid brain of the fish as compared to the non-alcoholic control group (Figure 3). Pretreatment with *Nux vom* 200c significantly increased the enthalpies or level of free water molecules as compared to the untreated alcoholic control group (Figure 3). However, the untreated non-alcoholic control group showed the highest level of enthalpies or free water molecules as compared to both the alcoholic groups, treated and untreated (Figure3).

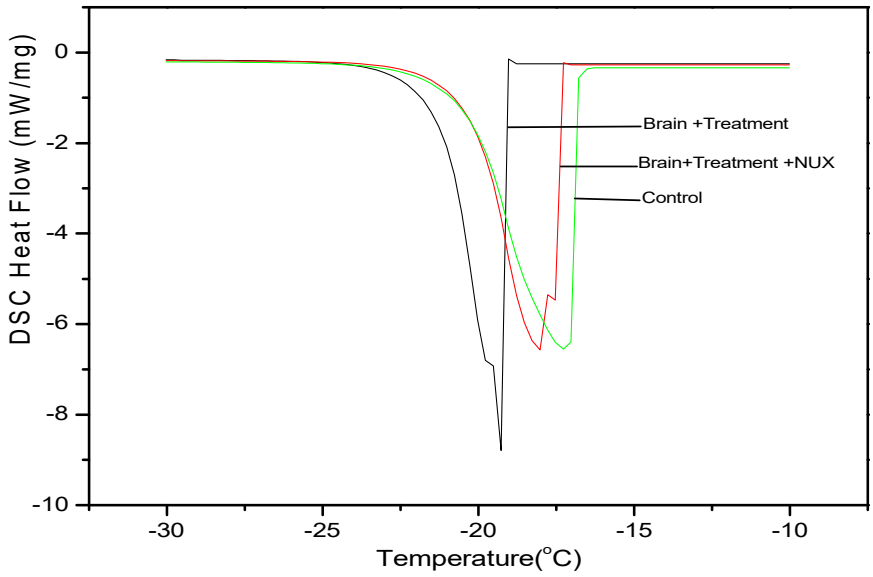

Fig 1: DSC curve showing freezing points of brain of *Channa* sp with three treatments.

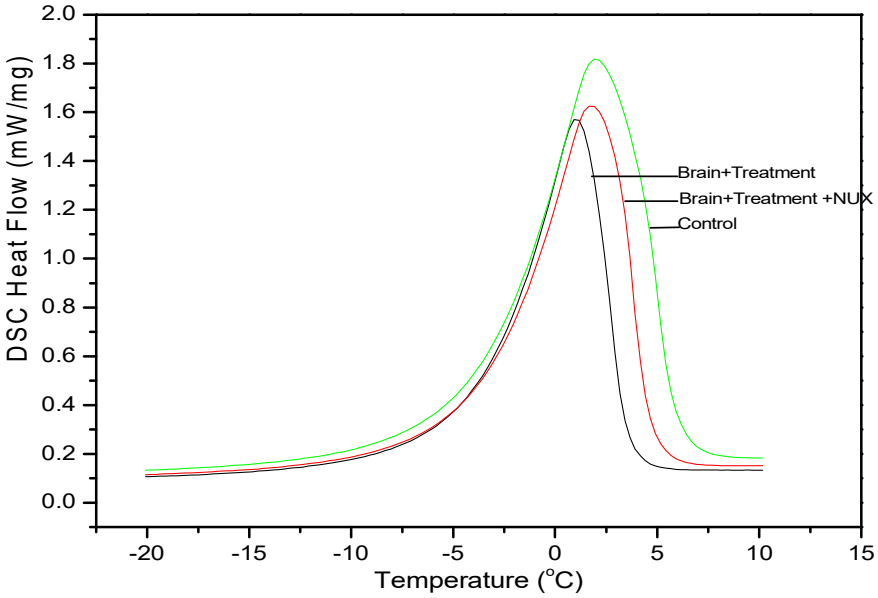

Fig 2: DSC curve showing melting points of brain of *Channa* sp with three treatments.

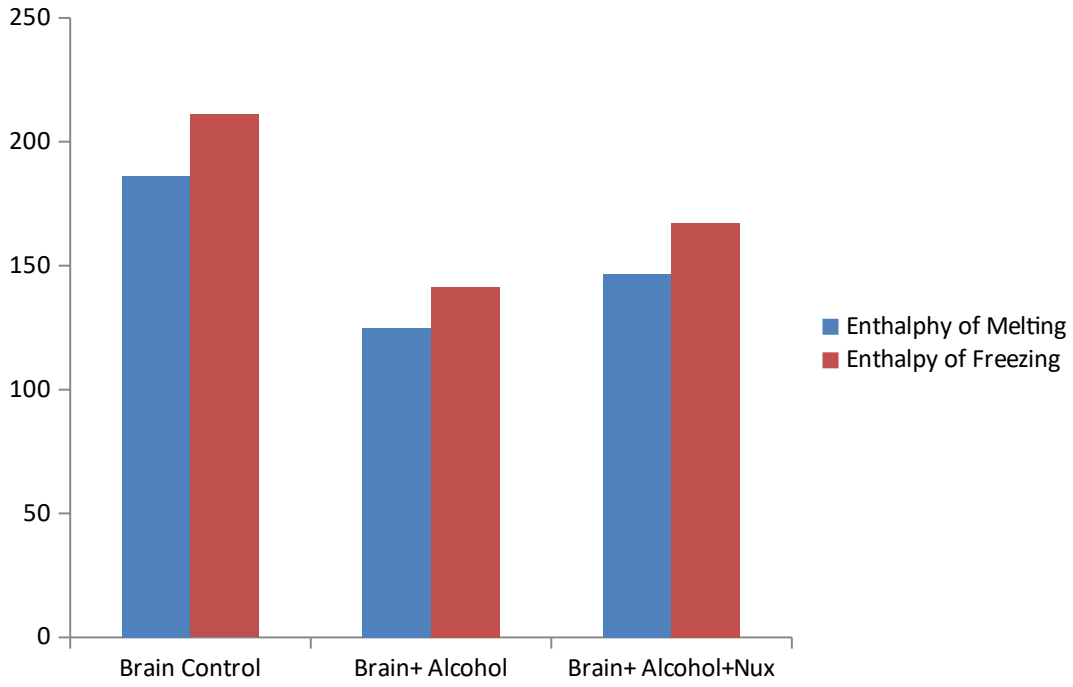

Fig 3: Histogram showing enthalpies of melting and freezing in brain of *Channa* sp .

Discussion

There is evidence that alcohol drinking induces neuroinflammation resulting in oedema of brain. Oedema linked neuroinflammatory pathways produce oxidative stress[14]. Water channel proteins Aquaporin 4 (AQP4) in glial cells play an important role in oedema of brain[7]. Diuretics like Furosemide and Acetazolamide have been reported to reduce ethanol induced high level of water content in rat brain[15,16]. This water content in the oedematous brain may include both free water and bound water. In our study we have measured indirectly the free water level in the brain of ethanol treated fish. Bound water, which does not freeze even at -100^{0}C, has been excluded from our experiment[11]. Here we see a significant reduction in free water molecules in the brain of ethanol treated fish (Figure 3). Our study further shows that *Nux vom* 200c increases the free water level significantly in the ethanol treated fish (Figure 3). In an earlier study we have observed that potencies of *Nux vom* could reduce ethanol intake in rats and also counter ethanol induced loss of righting reflex in rats and toads[7,17]. In an *in vitro* study it was observed that erythrocytes collected from an alcohol treated fresh water teleost contained less water as compared to those collected from control fish not exposed to alcohol. Again, *Nux vom* 30c significantly enhanced water influx in erythrocytes taken from the alcoholic fish. However, in this *in*

vitro tests free water molecules were not assessed separately. The authors suggested that *Nux vom* 30c might have acted on aquaporins on the erythrocyte membrane thereby enhancing water permeation[18].

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