FUNDAMENTAL RESEARCH

Altered solution structure of alcoholic medium of potentized Nux vomica underlies its antialcoholic effect

A Sukul[1], P Sarkar[1], SP Sinhababu[1] and NC Sukul[1]

Abstract

Nux Vomica 30c, 200c and 1000c were administered orally to three batches of albino mice for three days. Six hours after the last dose on the third day the mice were injected i.p. with ethanol 4g/kg body wt. They lost their righting reflex and lay motionless apparently sleeping due to alcohol. Mice treated with three potencies of Nux vomica regained their righting reflex more quickly than the corresponding untreated controls. Each of the three batches of mice was tested twice for ethanol sedation, once with a potency of Nux vomica and another time with a placebo control. The time interval between drug treatment and control was 10 days. NMR spectra of Nux 30, Nux 200, Nux 1000, alcohol 30, alcohol 30 (unagitated) and 90% alcohol showed significant difference from each other with respect to the spin-lattice relaxation time (T1) of the deuterium nuclei. This gives a measurable physical basis of the effective high potencies of Nux vomica. British Homoeopathic Journal (2000) 89, 73-77.

Keywords: Nux vomica; homoeopathic potencies; righting reflex; alcohol; sleep, NMR spectra; t_1 value.

Introduction

We have observed that the 30th centesimal potency of Nux vomica reduced alcohol induced sleep time in albino mice.[1] In our previous experiment we selected mice whose sleep time was close to a mean value. There is a wide individual variation in sleep time among the normal mice population. However, short sleep and long sleep mice lines, raised by selective breeding are often used in experiments on sleep induced with alcohol.[2,3] In the present study we examined the effect of three potencies of Nux vomica 30c, 200c and 1000c on the alcohol induced sleep time in the normal population of albino mice. In order to understand the physical basis of potencies, we also studied their NMR spectra.

An overdose of alcohol makes man and animals lose their righting reflex (RR) so that they lie motionless on the ground. Righting reflexes maintain the normal erect posture of an animal and operate through a series of responses which are integrated mostly in the nuclei of the mid-brain.[4] The efficacy of various anaesthetics is measured in terms of their effect on the duration of loss of righting reflex.[5,6] Nux vomica is known to counter the effects of alcohol on humans and rats.[7-10] The drug Nux vomica is prepared from the seeds of Strychnos nuxvomica L. The potencies of the drug are prepared as usual by successive dilution with 90% ethanol, 1:100 and succussion.[11]

Methods

Animals

Male Swiss albino mice (23-28g) purchased from the animal supplier Reeta Ghosh, Calcutta 54, were housed in groups of five in conventional cages with food and water ad libitum. They were kept in an animal house under natural light and rook temperature of 25±2°C. Experiments on mice were conducted at least two weeks after shipment.

Drugs used

The three potencies of Nux vomica, 30, 200 and 1000c, used in the present study were purchased in air-tight sealed tinted glass vials from King & Company, Calcutta. Each potency was mixed with sterile distilled water at the dosage of 0.05ml/2ml of water and administered orally to the mice at the rate of 0.05ml/mouse. The control consisted of 90% ethanol which was not successed. It was mixed with distilled water in the same proportion as the drug for oral administration to the mice.

[1]*Department of Zoology, Visva-Bharati University, Santiniketan 731 235, West Bengal, India*
(Reprints of article published in British Homoeopathic Journal (2000) 89, 73-77)

Dehydrated absolute ethanol was purchased from Bengal Chemicals and Pharmaceuticals Ltd., Calcutta. An appropriate volume of deionised and double distilled water was added to dehydrated ethanol to make it 90%. It was kept in 10 ml screw-capped glass vials sealed with adhesive tape. Distilled water prepared in our laboratory was free from contaminants.

Righting reflex

The batches of albino mice, one for each potency, were used. Each batch consisted of 20 mice. Mice were given control solution orally at 0.05ml/mouse/day for 3 days between 1.00 and 12.00h. Six hours after last dose they were injected intraperitoneally with ethanol (25% v/v) at 4g/kg body weight. As the mice became immobile after ethanol injection due to loss of RR they were laid on one side. The loss of RR was measured in terms of the duration of sleep time in minutes.[1-2] As soon as a mouse regained RR and took a sitting posture it was turned upside down. Recovery was judged as complete when a mouse could right itself three times within 1 min.[1-2] Figure 1 shows the percentages of mice regaining righting reflex plotted against time after ethanol injection.

After a lapse of 10 days the same mice of one batch were first treated orally with Nux vom 30c at the same dose as the control solution for three consecutive days. Six hours after the last dose they were given i.p. injection of ethanol at the same dose (4g/kg body weight). Similar tests were conducted with Nux vom 200 and Nux vom 1000 on two more batches of mice. Thus each of the tree batches of mice served once as control and next time as the test being treated with a potency of Nux vomica. In the case of Nux vom 1000, the sequence of treatments with control solution and drug was same as in Nux vom 30. The sequence was reversed in the case Nux vom 200, where active treatment was given first, followed by control. Two mice died from the Nux com 30c batch during the interval between tests.

NMR spectra

The spin-lattice relaxation time (T_1 in msec) of the naturally abundant ^2H was measured in 90% alcohol, alcohol 30, alcohol 30 (unagitated), Nux vom 30, Nux vom 200 and Nux vom 1000 using a AMX-400 NMR spectrometer operating at 61.4MHz at 22°C. The same sweep widths (3846Hz) and number scans (16) acquired for each experiment were used. Alcohol 30 was produced by successive dilution with 90% alcohol (1:100) and sonication at 20 kHz for 30 sec in 30 steps without using any drug at the initial step. Alcohol 30 (unagitated) was produced by successive dilution in 30 steps in a similar manner without any mechanical agitation. The preparation was kept in 10-ml screw-capped air-tight glass vials sealed with adhesive tape. The mechanism by which excess spin energy of a nucleus (here ^2H) is shared with the surroundings is referred to as the spin lattice relaxation. The time taken for a fraction 1/e = 0.37 of the excess energy to be dissipated is called the relaxation time.[12] T1 values of ^2H of water, hydroxyl, methylene and methyl groups of ethanol were measured from the stacked spectra with the help of a computer. Unlike proton, which has a spin half, deuterium has spin 1 and hence has an electric quadrupole moment in addition to its magnetic dipole moment. The interaction of the nuclear quadrupole moment with an electric field gradient provides a very efficient process for nuclear relaxation via the molecular rotation. Therefore, the deuterium T_1 measurement gives a straightforward estimation of the corresponding re-orientational correlation time.

Results

Righting reflex

The percentage of mice regaining RR following ethanol injection are shown in graphs for both controls as well as treatments in Figure 1 and Table 1. The effect of treatment with a potency of Nux was compared with that of the corresponding control by the chi-square test. Differences between the treatments and the corresponding controls were found to be significant ($P<0.01$, Figure 1, Table 1). The frequency of mice regaining righting reflex was always higher in the Nux-treated groups than in the corresponding controls.

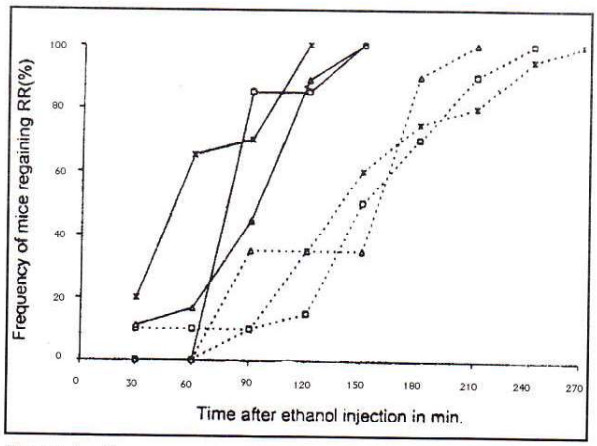

Figure 1 Frequency of mice regaining the righting reflex after i.p. injection of ethanol at 4 g/kg body weight. Mice were treated with *Nux vomica* 30c (△), *Nux vom* 200c (□) and *Nux vom* 100C (x) before ethanol administration. Differences between treatment (solid lines) and corresponding controls (dotted lines) are significant by (chi-square test ($P < 0.01$). For *Nux vom* 200, difference observed from 90 min onwards. $N = 20$ for *Nux* 200 and 1000 and $N = 18$ for *Nux* 30.

Table 1

Percentages of mice regaining righting reflex (RR) after i.p. injection of Ethanol at 4 g/kg body weight

Treatment		Percentage of mice regaining RR in time (min)								
	–	30	60	90	120	150	180	210	240	270
Control	–	0	0	35	35	35	90	100		
Nux 30	–	11.11*	16.67*	44.44*	88.89*	100*				
Control	–	10	10	10	15	50	70	90	100	
Nux 200	–	0	0	85*	85*	100*				
Control	–	0	0	10	35	60	75	80	95	100
Nux 1000	–	20*	65*	70*	100*					

Three batches of mice were treated orally each with Nux vomica 30c, 200c and 1000c before ethonol administration. Each of the three batches also severs as its untreated control. The time interval between treatment and control is 10 days. * Significant difference ($p<0.01$) by chi-squre tests between each pair of control for each potency of Nux.

NMR

The T_1 values with S.E. of ^2H of water, OH, CH_2 and CH_3 of ethanol in Nux 30, Nux 200, Nux 1000, 90% ethanol, ethanol 30 and ethanol 30 (unagitated) are presented in Table 2. The values (three replicates) were compared by ANOVA. 90% ethanol was non-drug unagitated control, ethanol 30 was the non-drug, agitated control. The T_1 value of H_2O increased significantly in ethanol 30 and Nux vom 30 but decreased in Nux 200 as compared to that of 90% ethanol (Table 2). The T_1 value of the OH group of ethanol increased significantly in Nux vom 30 and Ethanol 30 and Nux 1000 as compared to that in 90% ethanol, ethanol 30 (unagitated) and Nux 200. However, no peak of the OH group of water was observed in case of Nux com 1000. The T_1 value of CH_2 was highest in ethanol 30 (unagitated) followed by 90% ethanol, Nux 1000, Nux 30 and ethanol 30. Ethanol (90%), ethanol 30 and ethanol 30 (unagitated) differed significantly from each other with respect to the T1 value of ^2H of all the four chemical groups. Chemical shifts show some variation with respect to deuterium of water and OH of ethanol (Table 2).

The NMR spectrum of 90% ethanol, ethanol 30, ethanol 30 (unagitated), Nux vom 30 and Nux vom 200 shows four peaks in each case representing the ^2H nuclei of H_2O, OH, CH_2 and CH_3 of ethanol. The water hydroxyl peak in Nux 1000 is missing (Figure 2).

Table 2

Spin lattice relaxation time (T1 value of 2H of 90% ethanol, ethanol 30, ethanol 30 (unagitated), Nux vom 30, Nux 200, Nux 1000

		Alcohol		
Drug	Water	OH	CH2	CH3
90% Ethanol	110.794±1.166[a] (4.95)	106.905±0.883 [a] (5.65)	896.478±1.051[a] (3.93)	822.574±0.416[a] (1.50)
Alcohol 30	115.497±0.522[b] (5.02)	109.031±0.607 [b] (5.73)	857.894±0.692[b] (3.99)	802.692±0.292[b] (1.56)
Alcohol 30 (unagitated)	122.825±1.004[c] (4.96)	74.866±0.685 [c] (5.66)	953.692±0.406[c] (3.95)	819.739±0.567[c] (1.53)
Nux vom 30	123.375±1.007[c] (4.97)	111.825±0.455[d] (5.69)	872.914±0.797[d] (3.96)	839.729±0.526 [d] (1.52)
Nux vom 200	102.675±1.361[d] (4.58)	93.327±0.871 [e] (5.31)	827.414±0.339[e] (3.56)	790.593±0.44[e] (1.13)
Nux vom 1000		128.241±1.061[f] (5.52)	879.640±0.613[f] (3.95)	839.665±0.757[d] (1.53)

Measurements were taken by AMX-400 NMR operating at 61.41 MHz at 22°C. Three replicates.
[a,b,c,d,e,f] Significant difference by ANOVA (one way) in a column ($P<0.01$). Values in parentheses represent chemical shifts in ppm.

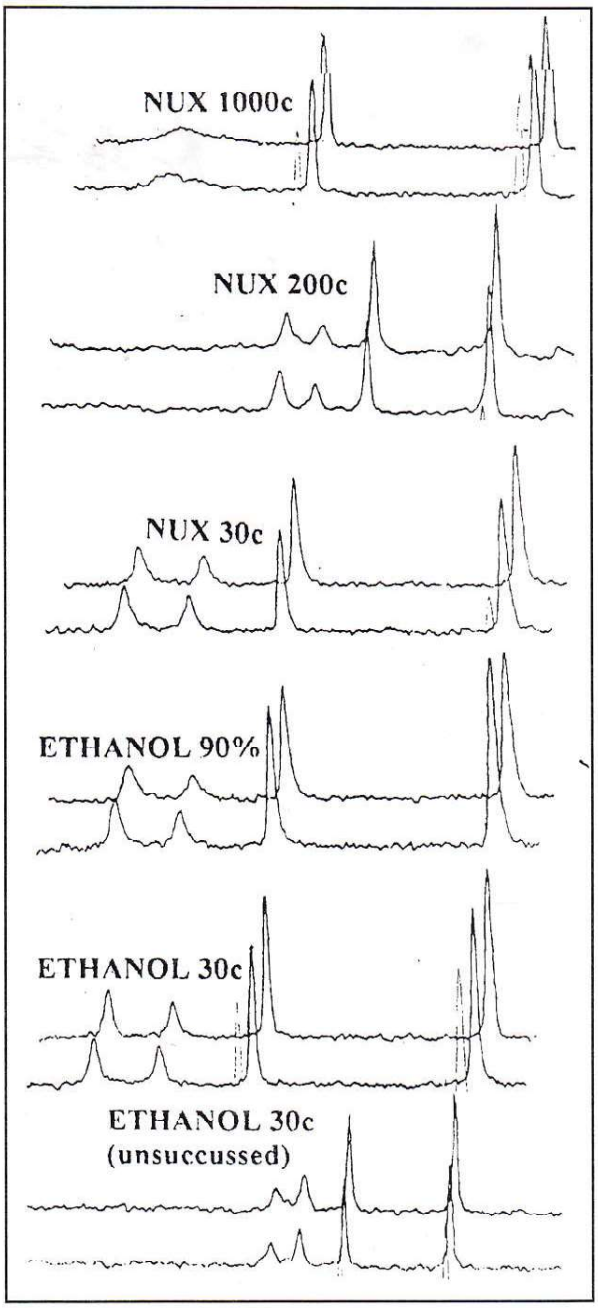

Figure 2 Part of the stacked spectra of ethanol 30c (unagitated), ethanol 30c, ethanol 90%, *Nux* 30c, Nux 200c and *Nux* 1000c.

Discussion

All the mice from the three batches, when treated with a potency of Nux, regained RR between 120 and 150 min. The same mice, when treated with a placebo, took between 210 nd 270 min to regain RR. The difference in total time for 100% recovery between treatment and corresponding control was 60 min with Nux 30, 90 min with Nux 200 and 150 min with Nux 1000 (Table 1, Figure 1). Thus treatment with Nux reduced alcohol induced sleep time in mice, and the higher the potency the shorter was the sleep-time relative to the corresponding control. The recovery rate of RR increased gradually following ethanol administration in mice without Nux vom. IN all the Nux-treated batches the rate increased 90 min after alcohol administration (Figure 1, Table 1). The results confirm our earlier observation on the anti-alcoholic effect of Nux vom 30c relating to the loss of RR.[1]

Like all quadrupolar nuclei, deuterium's relaxation is very sensitive to the effective correlation time, t_c of the vector, that joins the nuclei. t_c is the average time taken to rotate through 1 radian or roughly the reciprocal of the rate of tumbling in solution of the relevant piece of the molecule. Presence of paramagnetic molecules, such as transition metal ions or dissolved oxygen, affects T_1 values. Since ethanol is produced by repeated distillation and stored in glass containers, the presence of metal ions is a remote possibility. If they are present they would be equally distributed in all the drug items tested. The contribution of dissolved oxygen in organic solvents is normally of the order of 0.1 s^{-1}. Its effect will be insignificant in large molecules that relax rapidely.[13] Dissolved oxygen would also be present in equal quantities in all the samples, agitated or unagitated. Agitation would only make a marginal increase in dissolved oxygen in the liquid medium for a very brief period. Since the drugs and controls used had the same concentration (90%), the effect of concentration on T_1 is ruled out.

The difference in T_1 values in 90% ethanol and ethanol 30 and ethanol 30 (unagitated) of the four quadrupole nuclei suggests that mechanical agitation, as well as successive dilution, has contributed to the altered solution structure in ethanol 30. It appears from the chemical shift values (shown in parentheses in Table 2) that the deuterium nuclei are being shielded to a different extent. This in turn also influences T_1 values. Demangeat et al[14] reported an increased 4 MHz proton relaxation time (T_1) in high dilutions of a silica/lactose mixture as compared to control (0.9% NaCl). Haseba et al[15] measured that T_1 values of the naturally abundant ^2H of water molecules ($^2HO^2H/-O^2H$) in pure water and aqueous ethanol solutions. Samples of the aqueous ethanol solutions were subjected to ultrasonication. They showed that weak ultrasonication increased T_1 at all the concentrations measured and concluded that sonicated ethanol was more compact and homogeneous that unsonicated one. Further, the thermal motion of water molecules was greater in sonicated than in unsonicated ethanol. Our results also show a similar increase of T_1 value in sonicated ethanol with respect to water molecules. However, the T_1 decreased in sonicated ethanol with respect to the methyl group of ethanol as compared to that

in unsonicated ethanol. Our study included successive dilution and sonication of ethanol 30. T_1 of all the four ^2H nuclei in Nux vom 30 was higher than that in ethanol 30. Obviously, this difference is the result of introduction of Nux vom Q at the initial step of dilution. The difference in T_1 values between Nux vom 30 and Nux vom 1000 might be the result of added agitation and dilution. We have observed that T_1 values of the deuterium nuclei showed marked difference between Cina 1000c and 90% ethanol.[16] The absence of peak of hydroxyl deuterium of water in Nux 1000 is due to rapid exchange of ^2H between water and OH of ethanol. The two peaks merged resulting in a lower chemical shift (Table 2). The NMR data show that addition of a small quantity of a drug followed by successive dilution and mechanical agitation could bring about a significant change in the solution structure of the diluent medium such as aqueous ethanol. This altered solution structure in aqueous ethanol is thought to be responsible for the antihypnotic effect of the three potencies of Nux vomica. Haseba et al[15] also observed a marked change in the biological reactivity of sonicated ethanol.

Acknowledgement

We thank the Director, Sophisticated Instruments Facility, Indian Institute of Science, Bangalore for providing the NMR data of our samples.

References

1. Sukul A, Sinhababu SP, Sukul NC. Reduction of Ethanol induced sleep time in albino mice by potentized Nux vomica prepared with 90% ethanol. *Br Hom J.*1999; 88: 58-62.
2. Beauge F, Kerfriden G, Menez JF, Aufrere G, Bourhis B Le. Synaptic membrance responses to acute and chronic Ethanol intoxication in high Ethanol sensitive (HAS) and low Ethanol sensitive (LAS) selectively bred rats. *Ethanol Ethanolism* 1994; 29: 745-750.
3. Aufrere G, Menez FE, Beauge F, Duffy O, Bourhis B Le.ance and dependence in high Ethanol sensitivity (HAS) and low Ethanol sensitivity (LAS) rats after chronic Ethanol intoxication. *Ethanol Ethanolism* 1994; 29 397-410.
4. Ganong WF. *Review of Medical Physiology* 14th edn London: Prentice Hall International Inc, 1989; 172-173.
5. Alifmoff JK, firestone LL, Miller KW. Anaesthetic potencies of primary alkanols; Implications for the molecular dimensions of the anaesthetic site. *Br J Pharmacol* 1989; 96: 9-16.
6. Downes H, Courogen P M. Contrasting effects of anesthetics in tadpole biossays. *J Pharmacol Exp Ther* 1996; 278: 284-296.
7. Kent JT. *Homoeopathic material medica* 1911. Calcutta: Seth Dey & Co, 1962; 740-746.
8. Boericke W. *Pocket manual of homoeopathic material medica* 1927. Calcutta: Seth Dey & Co, 1976; 475-478.
9. Paul A, Sinha Babu SP, Sukul NC, Kurzina NP, Batuev AS. Effect of a potentized homoeopathic drug, Nux vomica on alcoholic rats and their hypothalamic neurons. *Proc Zool Soc, Calcutta* 1992; 45(Suppl A): 311-314.
10. Batuev AS, Sukul NC, Kurzina NP, Shchiolkina A. A new method for correcting the Ethanol motivation (in Russian). *Sechenov Physiol J* 1995; 81: 135-141.
11. Anonymous. *The American Homoeopathic Pharmacopoeia Philadelphia*: Boericke and Tafel. 9th edn. 1920; 15-19.
12. Banwell CN, McCash E. *Fundamentals of molecular spectroscopy*, 4th edn. New Delhi: Tata McGraw Hill Publishing Co. Ltd.; 1994; 212-213.
13. Sanders JKM, Hunter BK. *Modern NMR Spectroscopy.* Oxford: Oxford University Press; 1993; 160-164.
14. Demangeat JL, Demangeat C, Gries P, Poitevin B and Constantinesco A. Modifications des temps de relaxation RMN a 4 MHz des protons du solvent dans les tris hautes dilutions salines de silica/lactose. *J Med Nucl Biophys* 1992; 16: 135-145.
15. Haseba T, Matsushita K, Asakura T, Kameyama K, Tamaki T, Okouchi S, Watanabe T, Uedaira H. Diminution of biological reactivity of ethanol by changing the solution structure by weak ultrasonication. *Ethanolism Clin Exp Res* 1993; 17: 963-967.
16. Sukul Nirmal C, Sukul Anirban. Potentized Cina reduces root-knot disease of cowpeas. *Environ Ecol* 1999; 17: 269-273.