Transfer of the anti-alcoholic effect of *Nux vomica* 200 cH through water from one group of toads to another under alcohol anesthesia

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ABSTRACT

Background: A common practice is to give homeopathic medication to nursing mothers to treat their children, assuming that the drug will be conveyed by the milk. In the case of conventional treatment, the drug molecules are indeed passed on from the mother to her breastfed infant. However, high dilutions (HD) above 12 cH, i.e., over Avogadro's number, are traditionally held to lack any molecule from the starting material. If that is the case, then, does medication taken by the mother actually reach the child? To answer to that question, we developed animal models and demonstrated the transfer of HD effects between 2 groups of animals. **Aims:** To demonstrate the transfer of HD effects in an animal model in a much shorter time. **Methods:** Two batches of toads were respectively placed in two beakers, one containing *Nux vomica* 200 cH diluted with water 1:500 (direct treatment), and the other the same amount of distilled water were connected by cotton thread soaked in water and encased in a polyethylene tube (connected group); a third batch of toads (control) were placed in a beaker with 90% ethanol diluted with distilled water 1:500; all the animals were left 30 minutes, and then transferred to 3 independent beakers containing 209 mM ethanol. Every 10 minutes, the motionless toads were removed from the beakers, and placed on supine position, failure to recover the upright position after 60 sec was considered as loss of the righting reflex (RR). The experiment was replicated using large adult toads. **Results:** The percentage of toads losing the RR increased with the time of exposure to 209 mM ethanol in the 3 groups of toads. Significant difference in the percentage distribution was found between the control and the direct treatment and connected groups on χ^2 test ($p < 0.001$, $p < 0.01$, respectively), whereby the latter required much longer time to lose the RR, and did not differed between them. In the experiment with large adult toads, the control group lost RR in 78 min, whereas the 2 treated groups did not lose RR even after 240 min. **Conclusion:** *Nux-v* 200 cH countered the hypnotic effect of alcohol in young toads, and this effect was transferred through capillary water in the cotton thread, supporting the transfer of the effect of homeopathic medication from mother to child.

Keywords: *Nux vomica* 200 CH; transmission effect; alcohol; righting reflex; toads.

Introduction

A common practice is to give homeopathic medication to nursing mothers to treat their children, assuming that the drug will be conveyed by the milk. In the case of conventional treatment, the drug molecules are indeed passed on from the mother to her breastfed infant [1]. However, homeopathic preparations above dilution 12 cH, i.e., over Avogadro's number, are traditionally held to lack any molecule from the starting material. If that is the case, then, does medication taken by the mother actually reach the child?

We were able to demonstrate transfer of HD effects between individuals in plant models [2,3], where the effects were measured as protein expression and repression after several hours. The aim of the present study is to investigate the transfer of HD effects between individuals in an animal model over a much shorter time.

Young toads and tadpoles have long been used to ascertain the efficacy of different anesthetics used in surgery and other medical procedures [4 -6]. For that purpose, one of the effects assessed is the loss of the righting reflex (RR) [7]. The RR maintains the normal upright posture of animals through a series of responses that are mostly integrated in the mid-brain [8].

Homeopathic medicine *Nux vomica* is known to counter the effects of alcohol in human beings [9, 10]. *Nux-v* 30 cH reduced the loss of RR induced by alcohol in young toads [11] and *Nux-v* 200 cH produced a similar effect in toads [12]. Endler observed that dilutions of thyroxine exerted significant influence on the metamorphosis of tadpoles, which shows these animals serve as a suitable animal model for the study of homeopathy preparations. [13,14,15]

Materials and methods

Young toads, *Duttaphrynus melanostictus*, were freshly collected from ponds in the rural areas of Howrah district, West Bengal during the post-monsoon period in 2012. The animals were subjected to fasting for 15 hours to reduce regurgitation, which might contaminate the anesthetic solution. The anesthetic solution used was 209 mM ethanol, which is suitable to induce loss of RR in a relatively short time in young toads [5, 16].

Selected toads weighing 0.22-0.55 g were first treated with *Nux-v* 200 cH (Dr. Reckeweg, Germany) in 90% (v/v) ethanol. The drug was diluted with sterile distilled water 1:500 and placed in a one-liter beaker (direct treatment). An equal amount of sterile distilled water was placed in another beaker (connected treatment), and both were connected by a sterile 3-mm thick cotton thread soaked with distilled water and encased in a flexible 35-cm long polyethylene tube, except for the ends (Figure 1).

A batch of toads (n = 30) was half-immersed in each beaker for 30 minutes. Next, they were removed, and washed three times with sterile water. A third batch of toads (n = 30) (control) was placed in a beaker containing the control solution, 90 % ethanol diluted with distilled water 1:500, also for 30 min, and then washed three times with water.

Next, the three batches of toads were half-immersed separately in beakers containing 209 mM ethanol solution. (Figure 2). The animals that stopped moving upon being touched were removed from the beakers, and placed in supine position on a dry felt surface (Figure 3). Failure to straighten up within a cutoff time of 60 sec was considered as loss of RR [11, 17]. The toads exhibiting loss of RR were washed in water and isolated in a separate container.

The experiment was repeated with adult toads (45 – 53 g), comprising 20 animals per batch (directed, connected treatment and control)

The experiment was conducted over 8 days, at room temperature of $(30 \pm 1)^\circ \text{C}$. The pH of the ethanol solution was 8.47. The total number of toads in each group that lost RR at each interval of observation was recorded and subjected to statistical analysis. At the end of the study, the animals were released back into their natural habitat.

Fig. 1: The glass beaker on the left depicts the control group, placed in 90 % ethanol diluted with distilled water 1:500; middle beaker contains the toads that were directly treated with *Nux vomica* 200 cH diluted with distilled water 1:500; the beaker on the right contains toads immersed in distilled water, and connected to the middle beaker by a wet cotton thread encased in a polyethylene tube.

Fig. 2: Close-up of the toads half immersed in 209 mM ethanol solution.

Fig. 3: The toads were placed in supine position on a piece of paper to test loss of righting reflex induced by alcohol.

Results

The graphics in Figure 4 depicts the percentage of toads that lost the RR in each group as a function of the time of exposure to the ethanol solution. The differences in the percentage distributions were tested by means of the χ^2 test, the results are described in Table 1.

Fig. 4: Loss of RR during anesthesia with 209 mM ethanol was significantly delayed from 10 min onwards in the young toads treated with *Nux vomica* 200 cH ($p < 0.001$ on χ^2 test) and also in the group connected to the drug treated through water ($p < 0.01$ on χ^2 test) compared to control.

Table 1: Chi-square (χ^2) values at 5 % significance level of the control group, direct treatment with *Nux vomica* 200 cH, and the group connected to the direct treatment group.

χ^2 values			Significance		
Control vs. Drug Treated	Control vs. Connected	Drug Treated vs. Connected	Control vs. Drug Treated	Control vs. Connected	Drug Treated vs. Connected
8.14 (N= 60)	5.56 (N=60)	0.28 (N=60)	p < 0.001	p < 0.01	Not significant

The control group lost the RR in the shortest time, 70 min. Both the direct treatment and connected groups required significantly longer time, 180 and 140 min, respectively, to lose RR (Fig. 4). The percentage of toads that lost RR increased with the time of exposure to the ethanol solution. That increase was significantly higher in the direct treatment group compared to the controls (p < 0.001, Table 1), and also in the connection group compared to the controls (p < 0.01). The direct treatment and connected groups did not differ in this regard (Table 1). The large adult toads in the untreated control group lost RR in 78 min, the ones treated directly with *Nux-v* 200 cH and the connected group did not lose RR even after 240 min, when the experiment was finished.

Discussion

The amphibian skin is a semipermeable membrane that allows for the absorption of substances. Toads have both pulmonary and cutaneous respiration, and the latter is sufficient to prevent clinical hypoxia during anesthesia. Ethanol is not liable to ionization, and its binding to plasma proteins seems to be negligible [18,19]. The amphibians can tolerate very high concentrations of anesthetics including ethanol compared to mammals [5]. Thus, toads represent a good animal model to study the anti-alcoholic activity of high-diluted drugs, such as *Nux-v*.

High-diluted drugs are thought to be specifically structured water with strong hydrogen bonds that carries the information of the starting material. The higher the dilution, the stronger is hydrogen bonds, due to the cumulative effect of mechanical agitation [20]. When such dilutions come into contact with a sample of water, they might convey their own specific structure to the latter, which might thus produce the same therapeutic effects. *Nux-v* 200 cH absorbed through the naked skin of toads might have influenced the global molecular network (GMN) of the water pervading all the animals' tissues, and thus produced the anti-hypnotic effect, by an alteration of the GMN of water in the brain tissue. Neurosteroids produced *de novo* in the brain seem to influence the sensitivity to ethanol in specific brain regions, and the response to specific behavioral tests. Neurosteroids able to cross the blood-brain barrier are ubiquitous modulators of inhibitory neurotransmission [21]. High-diluted *Nux-v* might modulate the production of neurosteroids in the brain, thereby altering the action of ethanol on the brain. The present study suggests that a homeopathic HD might be able to transform the structure of water. The possibility of water transfer from one beaker to another is ruled out, because in that case water would have to cross a cotton thread already full of water before it was used to connect the beakers.

Conclusion

Nux vomica 200 cH induced anti-alcoholic effect in young and adult toads, measured as reduction of the loss of the righting reflex. This anti-alcoholic effect was transferred from directly treated toads to another group in another container connected to the former by the water soaking a cotton thread. Water thus serves as carrier of the information of a HD water structure, converting it into an active one. The results support the hypothesis of transfer of HD effects from mother to child.

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Transferência do efeito antialcoólico de *Nux vomica* 200 cH através de água, de um grupo de sapos para outro sob anestesia alcoólica

RESUMO

Introdução: A prescrição de medicamentos homeopáticos às mães de lactentes é uma prática habitual, sob o pressuposto de que a droga é transmitida pelo leite materno. No caso do tratamento convencional, moléculas da droga são efetivamente transmitidas de mãe para filho. Porém, tradicionalmente se considera que altas diluições (AD) acima da 12 cH, ou seja, acima do número de Avogadro, não conservam qualquer molécula da substância original. Nesse caso, será que a medicação tomada pela mãe alcança realmente a criança? Para responder essa questão, desenvolvemos um modelo animal e demonstramos a transferência dos efeitos de AD entre 2 grupos de animais. **Objetivos:** Demonstrar a transferência dos efeitos de AD num modelo animal, num período de tempo mais breve. **Métodos:** Dois lotes de sapos foram respectivamente colocados durante 30 minutos em 2 béqueres, um contendo *Nux vomica* 200 cH diluído 1:500 em água destilada (tratamento direto) e o outro, a mesma quantidade de água destilada (grupo conectado) conectados através de fio de algodão molhado com água e coberto por um tubo de polietileno; um terceiro grupo de sapos (controle) foi colocado num béquer com solução de etanol 90% em água destilada. Os animais foram após transferidos para 3 béqueres, respectivamente, contendo etanol a 209 mM. A cada 10 minutos, os sapos imóveis eram removidos dos béqueres e colocados em posição supina. Aqueles que não recuperaram a posição ereta em até 60 segundos foram considerados como tendo perdido o reflexo de endireitamento (RE). O experimento foi replicado com sapos adultos. **Resultados:** A percentagem de sapos que perderam o RE aumentou com o tempo de exposição ao álcool, nos 3 grupos. Foi observada diferença significativa entre o grupo controle e os grupos de tratamento direto e conectado no teste de χ^2 ($p < 0,001$, $p < 0,01$, respectivamente), sendo que os últimos precisaram de maior tempo para perder o RE, sem diferença entre ambos. Nos sapos adultos, o grupo controle perdeu o RE em 78 minutos, enquanto os 2 grupos tratados não perderam o RE depois de 240 min. **Conclusão:** *Nux-v* 200 cH inibiu o efeito hipnótico do álcool nos sapos, e este efeito foi transmitido pela água capilar no fio de algodão, dando suporte à hipótese de que o efeito dos medicamentos homeopáticos é transferido da mãe para o filho.

Palavras-chave: *Nux vomica* 200 CH; efeito de transmissão; álcool; reflexo de endireitamento; sapos.

Transferencia del efeito antialcoólico de *Nux vomica* 200 cH a través del agua, desde un grupo de sapos para otro, bajo anestesia alcohólica

RESUMEN

Introducción: La prescripción de medicamentos homeopáticos para las madres de niños es una práctica normal, bajo el presupuesto de que la droga se transmite a través de la leche materna. En el caso del tratamiento convencional, las moléculas de la droga son efectivamente transmitidas de la madre al niño. Sin embargo, tradicionalmente se ha considerado que las altas diluciones (AD) por encima de 12 cH, es decir, por encima del número de Avogadro, no conservan ninguna molécula de la sustancia original. En ese caso, ¿el medicamento tomado por la madre llega realmente a la niña? Para responder a esta pregunta, hemos desarrollado un modelo animal para demostrar la transferencia de los efectos de AD entre 2 grupos de animales. **Objetivos:** Demostrar los efectos de la transferencia del efecto de AD, en un modelo animal, en un período más corto de tiempo. **Métodos:** Se prepararon dos lotes de sapos y se los colocaron respectivamente durante 30 minutos en dos vasos, uno que contenía *Nux vomica* 200 cH diluido 1:500 en agua destilada (tratamiento directo) y el otro, la misma cantidad de agua destilada (grupo conectado) conectado a través de un cable de algodón humedecido con agua y cubierta por un tubo de polietileno. Un tercer grupo de sapos (control) se colocó en un vaso de lleno con solución de etanol al 90% en agua destilada. Los animales fueron transferidos para tres vasos, con solución de etanol a 209 mM. A cada 10 minutos, los sapos inmóviles eran sacados de los vasos y colocados en la posición supina. Aquellos que no se han recuperado la posición vertical dentro de 60 segundos se consideró que han perdido el reflejo de enderezamiento (RE). El experimento se repitió con sapos adultos. **Resultados:** El porcentaje de los sapos que han perdido el RR aumentó con el tiempo de exposición al alcohol, en los 3 grupos. Diferencia significativa entre el control y los grupos de tratamiento y conectado en directo χ^2 test ($p < 0,001$, $p < 0,01$, respectivamente), mientras que el segundo necesita más tiempo para perder el RE, sin diferencia entre ellos. En sapos adultos, el grupo perdió el control RE en 78 minutos, mientras que los 2 grupos de tratamiento no perdió el RE después de 240 min. **Conclusión:** *Nux-v* 200 cH inhibió el efecto hipnótico de alcohol en sapos, y este efecto fue transmitido por el agua capilar en hilo de algodón, que apoya la hipótesis de que el efecto de los remedios homeopáticos es transferido de la madre al niño.

Palabras clave: *Nux vomica* 200 CH; efecto de transmisión; alcohol; reflejo de enderezamiento; ranas.

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