

# Intergroup transfer of anti-alcoholic effect of *Nux vomica* 200 cH through the body of a live toad

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## ABSTRACT

**Background:** A homeopathic potency might often be given to nursing mothers for the treatment of their infants. Potencies above 12 cH exceed Avogadro's number and thus are too diluted so as to contain any original drug molecule. A potency is thought to be specifically structured water carrying the imprint of original drug molecules. It may induce changes in the water structure in the mother's body and reach the suckling infant through her milk. Using a toad model, we recently demonstrated that the anti-alcoholic effect of *Nux vomica* 200 cH could be transferred from one group of toads to another through capillary water carrying the information of *Nux-v*. Homeopathic potencies show UV spectra distinct from the hydro-ethanolic diluent medium. Does a potency remain effective even after passage through a living body? **Objectives:** To demonstrate that a potency effect might be transferred through the body of a live toad to other groups of toads connected to it through water. In addition, we sought to establish whether the UV spectra of the drug solution and the water connected to the drug are similar in nature. **Methods:** A live toad was held vertically with one hind limb dipped in *Nux-v* 200 cH solution in a beaker and another limb in distilled water in another beaker. The second beaker was connected by wet cotton threads encased in polythene tubes to 5 beakers, each one of which contained adult toads in distilled water. A batch of toads was directly treated with *Nux-v* 200 cH. An equal number of toads in distilled water served as untreated control. After 30 min, the control and the two batches of treated toads were kept separately in 209 mM ethanol solution. Toads that stopped moving were placed in supine position on a dry surface. Failure to assume a normal sitting posture within a cutoff time of 60 sec was regarded as loss of righting reflex (RR). The experiment was replicated using a large number of toads. UV spectra of *Nux-v* 200 cH solution and of water before and after connection with the drug were obtained. **Results:** The percentage of toads that exhibited RR loss in the 3 groups increased with the time of exposure to 209 mM ethanol solution. The loss of RR was significantly delayed in the group subjected to direct treatment ( $P < 0.001$ , chi-square test) and the connected group ( $P < 0.01$ ,  $\chi^2$  test) compared to the control. The two former groups did not differ from each other significantly. UV spectra of *Nux-v* 200 cH solution were similar to that of the water connected to the drug solution. **Conclusion:** The anti-alcoholic effect of *Nux-v* 200 cH could be transferred through the body of a live toad to other groups of toads. The drug did not undergo denaturation during its passage through the living body. The fact that water carries the information of the original drug was further evidenced by the spectral properties of the water connected to the drug solution through capillary water.

**Keywords:** *Nux vomica* 200 cH, UV spectra, transmission effect, toads, righting reflex.

## Introduction:

In a series of experiments on plants and animals, we demonstrated that the effect of a potentized homeopathic drug could be transferred from one individual to another through water [1-3]. Those studies point to the fact that water carries the imprint of a homeopathic potency. Homeopathic physicians have long noted that a breastfed infant responds to the therapeutic effect of a potency given to its mother. In this case, the potency must have passed through the mother's milk to the infant. It is assumed that the potency given to the mother might have changed the water structure in her body, including her milk, into its own form, and thus the mother's milk also carries the the potency imprint. Here we witness the trans-individual transfer of the potency effect from the mother to her baby. In the present study, we sought to establish whether the effect of a potency might be transferred through the body of a live toad to other toads, thus simulating the trans-maternal transmission of the drug effect. In an earlier study, we showed that the anti-alcoholic effect of *Nux vomica* 200 cH was transferred from one group of toads to another through water in capillaries [3]. That toad model was slightly modified here to suit the objective of the present study. Toads have long been used as a standard animal model to study the efficacy of different anesthetics used in surgery [4,5]. Toads have also served as suitable animal models for the study of homeopathic preparations [6,7].

Homeopathic potencies might be differentiated from their diluent medium, aqueous ethanol, by electronic spectra [8-14]. Using this technique we demonstrated that samples of water connected to a homeopathic potency, such as *Nux-v* 200 cH, in a different container show electronic spectra characteristic of the potency used in the study.

## Material and methods:

Experiments on toads: Adult toads *Duttaphrynus melanostictus* were freshly collected from rural areas of South 24 Parganas, West Bengal, India in November, 2012. The animals were thoroughly washed thrice in sterile water and allowed to fast for 24 hours to reduce regurgitation, which might contaminate the anesthetic solution. One toad was tied to a stiff plastic sheet by cotton threads to restrain its free movement. This toad was held vertically over a pair of 100 ml beakers in such a way that one of its hind limbs was dipped into *Nux-v* 200 cH diluted with distilled water 1:500 in a beaker. The other hind limb was dipped into an equal amount of distilled water kept in another beaker (Fig. 1). A small amount of Vaseline was applied around the base of the hind limbs to prevent seepage of liquid from one beaker to another over the body surface of the toad. *Nux-v* 200 cH (Dr. Reckeweg, Germany) was obtained from the market as an ethanol-water mixture in which ethanol content was 90%.



Fig.1: Toad held vertically against a stiff plastic sheet with one hind limb dipped into *Nux vomica* 200 cH diluted with water 1:500 in a beaker and another hind limb dipped in water in another beaker.

When a non-anesthetized toad is placed on its back, it turns over and assumes an erect posture in 0.5-0.8 sec. Under ethanol anesthesia, it is not able to present that response even in 60 sec. We examined the toads in each of the 3 groups at intervals to establish whether they had lost RR following application of the abovementioned method. The ones that showed to have lost RR were set aside, and their number was expressed as percentage at that time interval. The ones that turned over within 60 seconds at this time of the test were returned to the ethanol solution.

The second beaker containing water was connected by sterile 3-mm thick wet cotton threads encased in 35-cm long polythene tubes to 5 one-liter beakers, each of which contained 250 ml of distilled water. Each of these large beakers contained two toads half immersed in water (Fig 2).



Fig. 2: Toads half immersed in water in large beakers are connected through wet threads encased in polythene tubes.

Failure to right itself within a cut-off time of 60 sec was judged as loss of RR [3,12,15-17]. Toads showing loss of RR were washed in water and kept in a separate container. The experiment was conducted over 3 consecutive days with 90 toads (45.5 to 50 g) at room temperature ( $28 \pm 1$  °C). All the toads were released to their natural habitat after the experiment. The pH of the ethanol solution was 8.47.

An equal number of toads (control) was kept in 5 one-liter beakers, each one containing an equal amount of distilled water. The third batch served as direct treatment, whereby the toads were directly treated with *Nux-v* 200 cH solution 1:500. After 30 min, all the toads were removed and washed thrice with sterile water. The control and test groups were kept separately in 209 mM ethanol solution, 1.5 cm deep. The toads that stopped moving upon being touched were removed from the container and placed on supine position on a dry flat surface (Fig. 3).



Fig. 3: A toad placed on supine position on a tray to test the loss of righting reflex.

UV absorption spectra of the samples were taken in dual beam mode using a UV –VIS spectrophotometer (Perkin Elmer, Lamda 35). While a quartz cuvette contained the test sample, another contained distilled water only. An average of 3 consecutive spectra was taken for each sample with scan speed 400 nm/min. For undiluted *Nux-v* 200 cH, the second cuvette contained 90% ethanol only. Three 50-ml beakers were used, one contained *Nux-v* 200 cH diluted with distilled water 1:5 The other two (b and c) contained distilled water only. An aliquot of 'a' was taken in a cuvette and its UV spectrum was measured. Then 'a' was connected to 'b' by wet cotton thread for 5 sec. After disconnection, an aliquot from 'b' was taken and its spectrum was recorded. Then 'a' was connected to 'c' for 30 min. After disconnection an aliquot from 'c' was taken and its spectrum was recorded.

## Results

As the toads were exposed to the anesthetic solution, they became hyperactive initially trying to jump out of the container. This is the delirium phase of anesthesia and is quite normal in amphibians [18]. After a while, they became gradually quiet. The percentage of toads that lost RR in the 3 groups is shown in graphs against the time of exposure to the ethanol solution (Fig 4).

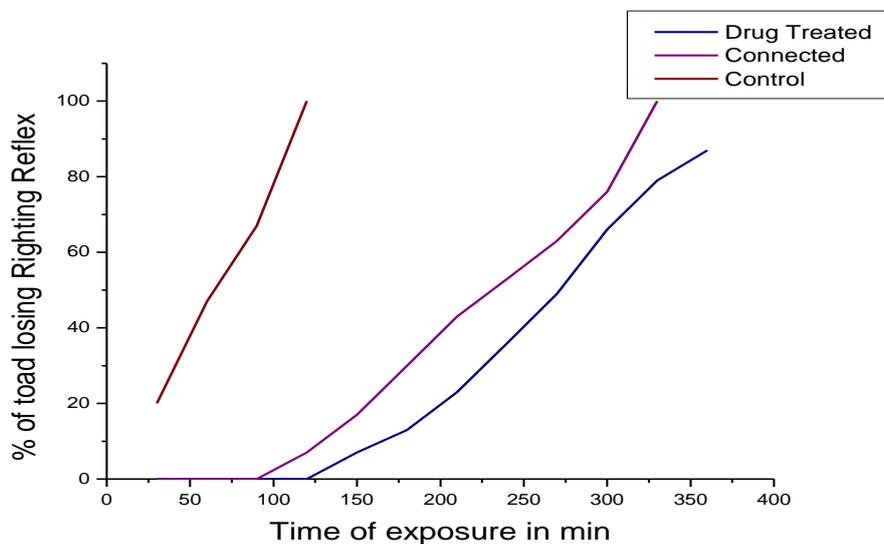


Fig. 4: Loss of RR during anesthesia with 209 mM ethanol was significantly delayed from 30 min onwards in the toads treated with *Nux vomica* 200 cH ( $p < 0.001$ ,  $\chi^2$  test) and also in the group connected via live toad through water ( $p < 0.01$ ,  $\chi^2$  test) compared to the control.

The differences in the percentage distributions were tested by means of  $\chi^2$  test and the results are presented in Table 1.

Loss of RR due to ethanol solution was significantly delayed in toads directly pretreated with *Nux-v* 200 cH ( $p < 0.001$ ) and also in the toads connected with capillary water through the body of a live toad ( $p < 0.01$ ) compared to the untreated controls (Table 1). There was no significant difference between the direct treatment group and the connection group relative to the time of loss of RR (Table 1). While the untreated control group lost RR in 120 min, the connected group did the same in 330 min. Only 86.7 % of the direct treatment group lost RR in 360 min, when the experiment was finished.

Table 1: Chi-square ( $\chi^2$ ) values at 5 % level of significance for control toads, a test group directly treated with *Nux vomica* 200 CH and another group connected to the direct treatment group. The actual number of toads that lost righting reflex (RR) within a fixed time of 300 min is given between parentheses. The number of toads in each group was 30.

$\chi^2$ values			Significance		
Control (30) vs. Drug Treated (20)	Control (30) vs. Connected (23)	Drug Treated (20) vs. Connected (23)	Control vs. Drug Treated	Control vs. Connected	Drug Treated vs. Connected
12 (N= 60)	7.92 (N=60)	0.72 (N=60)	p < 0.001	p < 0.01	Not significant

UV spectra (Perkin Elmer, Lamda 35) of undiluted *Nux-v* 200 CH showed a peak at 192 nm with absorbance intensity at 0.17 (Fig 5). The 1:500 dilution of *Nux-v* 200 cH showed a blue shift (Fig. 6) and lower intensity compared to the undiluted drug (Fig 5). The spectra of water before connection showed no absorbance, but the ones of water after connection with the drug at different intervals of time showed blue shift and absorbance between 0.3 and 0.4 (Fig 6).

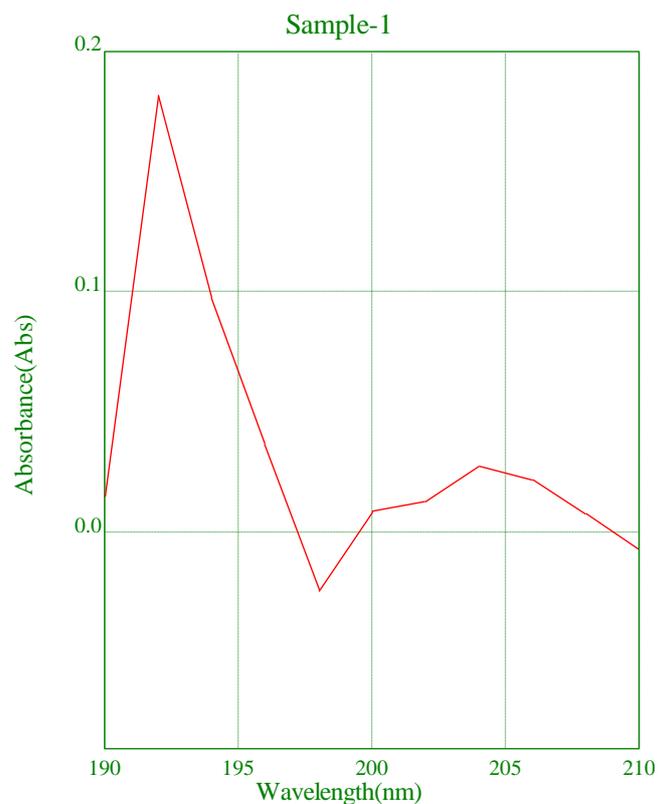


Fig. 5: UV absorption spectra of undiluted *Nux vomica* 200 cH

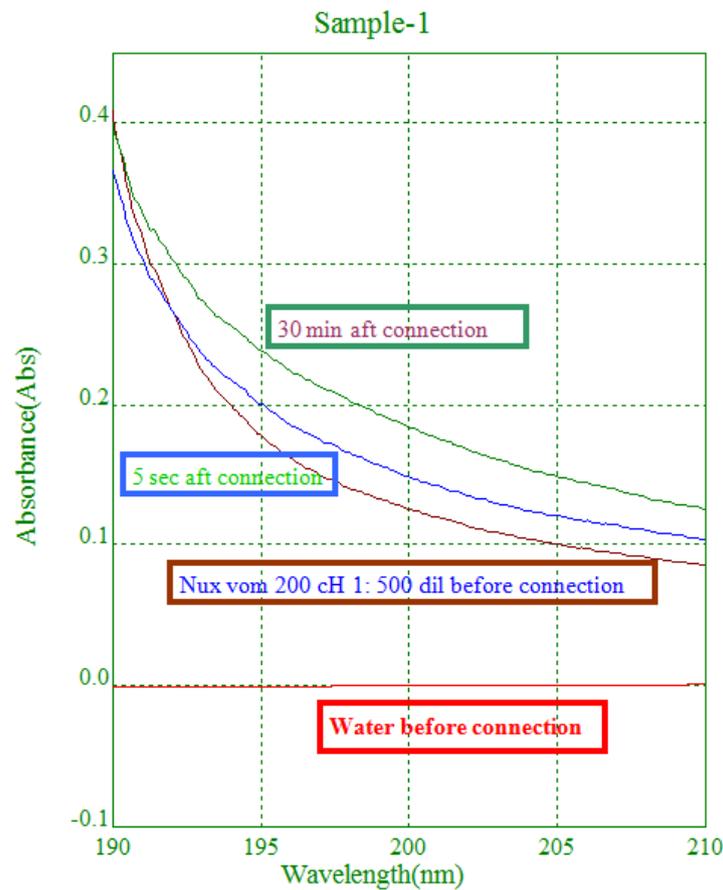


Fig. 6: UV absorption spectra of water before and after connection with *Nux vomica* 200 cH solution.

### Discussion:

The skin of toads shows functional properties similar to the distal mammalian nephron [19]. Nephron is the excretory unit of the kidney and is permeable to water and alcohol solution. The skin of the ventral surface and of the limbs of oads partly immersed in anesthetic solution absorbs the solution directly [20]. Ethanol, after absorption through the skin, interacts in a non-specific manner with phospholipid bilayers at the lipid-water interface of cell membrane. It alters the orientation of lipid head groups and modifies the function of many different proteins in the central nervous system membranes, thereby producing acute changes in many different cells and organs. Due to this non-specific interaction, large numbers of ethanol molecules are required to produce an intoxication effect [21]. Besides their effect on lipid bilayers, alcohol also interacts directly with integral membrane proteins [22]. The biological effect of alcohol, including anesthesia, might result from a combination of alcohol-induced changes in the cell membrane, as well as specific membrane protein-alcohol interactions [23].

A homeopathic potency such as *Nux-v* 200 cH is thought to be specifically structured water preserved by ethanol. It is assumed that after absorption through the skin, the drug modifies the structured water at the lipid-water interface, thereby reducing the anesthetic effect of alcohol [24]. After absorption through one live toad's hind limb, the potentized drug might modify the global molecular network (GMN) of water inside the toad's body, and thus, the water in contact with the other hind limb in the second beaker becomes specifically

structured and behaves as the potentized drug. From the second beaker, the drug message is transmitted through capillary water in wet threads to other beakers containing water and test animals. The toads in the connected containers thus got the treatment effect as observed in our earlier study [3].

The transmission of the drug effect through capillary water is further supported by the electronic spectra of water samples from the connected container. As the electronic spectra of water in the connected container showed similarity with that of the original drug solution in only 5 seconds, the possibility of actual transport of drug solution to the connected container is ruled out. Moreover, the capillaries in the connecting threads were already full of water before the connection was established. The present study further shows that water structures of the potentized drug do not undergo denaturation during the passage through the living body. This is another evidence in support of the potency effect on the suckling infant resulting from the treatment of his mother with a potentized drug.

### **Conclusion:**

*Nux vomica* 200 cH countered the effect of effect in toads and this effect could be transferred through the body of a live toad to other groups of toad connected by capillary water in wet cotton threads. UV spectra of *Nux vomica* 200 cH and of water connected to the drug by wet cotton threads showed similarity. The study provides evidence that water carries the imprint of potentized drugs.

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## Transferência intergrupo do efeito antialcóico de *Nux vomica* 200cH através do corpo de um sapo vivo

### RESUMO

**Introdução:** Frequentemente, para tratar um bebê alimentado com leite materno, o medicamento homeopático é administrado à mãe. As potências acima da 12 cH ultrapassam o limite de Avogadro e, portanto, não contém mais qualquer molécula da droga original. Uma potência é compreendida como água especificamente estruturada que carrega a impressão das moléculas originais da droga. Uma potência pode induzir alterações na estrutura da água no corpo da mãe e alcançar o bebê através do leite. Utilizando um modelo com sapos, recentemente demonstramos que o efeito antialcóico de *Nux vomica* 200 cH pode ser transferido de um grupo de sapos para outro através de água capilar com a informação de *Nux-v* 200 cH. As potências homeopáticas exibem espectros UV diferentes do diluente hidroetanólico. Será que uma potência permanece eficaz depois da passagem por um corpo vivo? **Objetivos:** Demonstrar que o efeito de uma potência pode ser transferido através do corpo de um sapo vivo para um outro grupo de sapos conectado a ele através de água. Além disso, procuramos estabelecer se os espectros UV da solução da droga e da água conectada à droga têm natureza similar. **Métodos:** Um sapo vivo foi mantido em posição vertical, com uma das patas posteriores submergida num béquer com solução de *Nux-v* 200 cH, e a outra pata posterior num béquer com água destilada. Esse segundo béquer foi conectado mediante fios de algodão umedecidos dentro de tubos de polietileno a 5 béqueres, cada um dos quais continha sapos adultos e água destilada. Um grupo de sapos foi tratado diretamente com *Nux-v* 200 cH; um número igual foi mantido em água destilada (controle). Trinta minutos depois, o grupo controle e os 2 grupos tratados foram separadamente colocados em solução de etanol 209 mM. Os sapos que cessaram de se movimentar foram colocados em posição supina sobre uma superfície seca. A impossibilidade de recuperar a postura ereta normal num tempo de 60 segundos foi considerado como perda do reflexo de endireitamento (RE). O experimento foi repetido com um número maior de sapos. Foram obtidos espectros UV da solução de *Nux-v* 200 cH e da água antes e depois da conexão com a droga. **Resultados:** A percentagem de sapos com perda do RE nos 3 grupos aumentou paralelamente ao tempo de exposição à solução de etanol 209 mM. A perda do RE foi significativa demorada no grupo submetido a tratamento direto ( $P < 0.001$ , chi-quadrado) e no grupo tratado por conexão ( $P < 0.01$ , chi-quadrado) por comparação ao grupo controle; os dois grupos tratados não apresentaram diferença significativa entre eles. O espectro UV da solução de *Nux-v* 200 cH foi similar àquele da água conectada à solução da droga. **Conclusão:** O efeito antialcóico de *Nux-v* 200 cH pode ser transferido através do corpo de um sapo vivo para outro grupo de sapos. A droga não sofreu desnaturação com a passagem através de um corpo vivo. O fato de que a água carrega a informação da droga original foi ainda evidenciado pelas propriedades espectrais da água conectada à solução da droga através de água capilar.

**Palavras-chave:** *Nux vomica* 200 cH, espectros UV, efeito de transmissão, sapos, reflexo de endireitamento.

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## Trasferencia entre grupos del efecto antialcohólico de *Nux vomica* 200 cH a través del cuerpo de un sapo vivo

### RESUMEN

**Introducción:** Frecuentemente, para tratar un bebé alimentado con leche materna, el medicamento homeopático es administrado a su madres. Las potencias superiores a la 12 cH superan el límite de Avogadro y por ende no conservan ninguna molécula de la droga original. Una potencia puede ser entendida como agua específicamente estructurada que conduce la impresión de las moléculas originales de la droga. Una potencia puede inducir alteraciones en la

estructura del agua en el cuerpo de la madre y llegar al bebé con la leche. Utilizando un modelo de sapos, recientemente demostramos que el efecto antialcohólico de *Nux vomica* 200 cH puede ser transferido de un grupo de sapos a otro a través de agua capilar impregnada de la información de *Nux-v* 200 cH. Las potencias homeopáticas manifiestan espectros UV diferentes del espectro del medio diluyente hidroalcohólico. ¿Se conserva la eficacia de una potencia homeopática después de pasar por un cuerpo vivo? **Objetivos:** Demostrar que el efecto de una potencia puede ser transferido a través del cuerpo de un sapo vivo a otro grupo de sapos conectados a éste mediante agua. Además, buscamos determinar si los espectros UV de la solución de droga y del agua conectada a ella son de naturaleza semejante. **Métodos:** Se colocó un sapo vivo en posición vertical, una de las patas posteriores fue sumergida en un matraz con solución de *Nux-v* 200 cH y la otra pata posterior en un matraz con agua destilada. El segundo matraz fue conectado mediante hilos de algodón humedecidos colocados en tubos de polietileno a 5 matraces con agua destilada y sapos adultos. Un grupo de sapos fue tratado directamente con *Nux-v* 200 cH; un número igual fue conservado en agua destilada (control). Treinta minutos después, el grupo control y los 2 grupos tratados fueron colocados por separado en solución de etanol 209 mM. Los sapos que dejaron de moverse fueron colocados en posición supina sobre una superficie seca; la imposibilidad de recuperar la postura erecta normal en un plazo de 60 segundos fue considerada como pérdida del reflejo de enderezamiento (RE). El experimento fue repetido con un número mayor de sapos. Fueran registrados los espectros UV de la solución de *Nux-v* 200 cH y del agua antes y después de ser conectada a la droga. **Resultados:** El porcentaje de sapos que presentó pérdida del RE en los 3 grupos aumentó paralelamente al tiempo de exposición a la solución de etanol 209 nM. La pérdida del RE presentó retraso significativo en los grupos tratados directamente con *Nux-v* 200 cH ( $P < 0.001$ , chi-cuadrado) y conectado ( $P < 0.01$ , chi-cuadrado) comparados al grupo control; los grupos tratados no presentaron diferencia significativa entre ellos. El espectro UV de la solución de *Nux-v* 200 cH fue similar al del agua conectada a la solución de droga. **Conclusión:** El efecto antialcohólico de *Nux-v* 200 cH fue transferido a través del cuerpo de un sapo vivo a otro grupo de sapos. La droga no sufrió desnaturalización al pasar a través de un cuerpo vivo. La propiedad del agua de conducir la información de la droga original fue también evidenciada por las propiedades espectrales del agua conectada a la solución de droga mediante agua capilar.

**Palabras-clave:** *Nux vomica* 200 cH, espectros UV, efecto de transmisión, sapos, reflejo de enderezamiento.



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