

# High dilutions of homeopathic remedies induce relaxation of rat aorta precontracted with Noradrenalin

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## ABSTRACT

Homeopathic potencies have been reported to produce alteration of contraction in isolated rat ileum in an organ bath. Potentized homeopathic drugs like *Lycopus V* and *Aurum met* are used for the treatment of hypertension. The purpose of this study is to see whether *Lycopus virginicus* y *Aurum metallicum* (30 cH) could produce relaxation of isolated rat aorta in the organ bath. The aorta of rats were dissected out, placed in Krebs-Henseleit solution, cleared of connective tissue and endothelium and cut into 2-2.5 mm long rings. The rings were fixed in organ baths with the upper end connected by a string to an isometric transducer which was finally attached through data acquisition equipment to a computer. *Aurum met* 30 CH, *Lycopus V* 30 CH, and their medium 90% ethanol were added separately to the bathing fluid containing the aorta rings which were precontracted with noradrenalin (NA) or norepinephrine (NE). Both the drugs produced significant relaxation of the aorta ( $p < 0.001$ ) precontracted with NA ( $10^{-7}$  M). The control did not show any marked effect on the NA induced contraction of aorta. The two potentized drugs used are too dilute to contain any original drug molecules. So, the usual pharmacological interaction between drug molecules and smooth muscle receptors of isolated aorta is ruled out. The exact mechanism is different but unknown. Both *Lycopus V* 30 CH and *Aurum met* 30 CH are effective in reducing NA induced contraction of rat aorta. Drugs can directly act on the isolated rat aorta without any systemic influence.

**Keywords:** High dilution drugs, isolated aorta, hypertension, noradrenalin.

## Introduction

Homeopathic remedies have been used for the treatment of patients over a couple of centuries. The drugs are also effective on animals and plants [1]. These are mostly *in vivo* studies where many factors may interfere with the actual drug effect. Isolated organs have been used for over a century to evaluate the pharmacological effect of drugs. These *ex vivo* models are independent of the systemic interference inherent in studies on live

animals [2, 3, 4]. In recent years pharmacological activity of plant extracts has been evaluated successfully on isolated organs [5, 6]. Van Wijk and Albrecht [7] reported four independent studies, conducted during the period from 1947 till 2002, providing evidence for the effect of homeopathic drugs on isolated rat ileums. Homeopathic remedies have been known to act on the vascular system. The drugs have been reported to act on the isolated ileum and duodenum of rats [8, 9]. Two homeopathic remedies like *Aurum metallicum* and *Lycopus V* have been used for the treatment of hypertension in man [10]. The purpose of the present study is to see whether these two drugs show any effect on the isolated rat aorta contracted with noradrenalin (NA).

## Materials and methods

Sprague-Dawley rats weighing 270 – 278 gm were sacrificed by stunning followed by cervical dislocation. The rats were maintained individually in rat cages on rat feed and water *ad lib* in an animal house. Research protocols and care of rats were based on the principles and guidelines adopted by the guide for the care and use of laboratory animals (NIH publication No. 85-23, revised in 1996) and approved by the local ethical committee of HuachiewChalermprakiet University, Thailand.

The aorta was quickly dissected out and placed in a petridish containing Krebs-Henseleit solution (119 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM MgSO<sub>4</sub>, 25 mM NaHCO<sub>3</sub> and 5.6 mM glucose). The aorta was cleared of connective tissue, fat and endothelium, and cut into rings 2-2.5 mm in length. Each vascular ring was fixed between two stainless steel hooks inside a 15 ml capacity organ bath containing the bathing solution. The organ baths were kept at a thermostatically controlled temperature of 37°C and gassed with 95% Oxygen and 5% Carbon dioxide. While one hook was fixed to the organ bath, the other to a 5g isometric transducer (Heto Lab Equipment BIOPAC System Scientific Promotion CO Ltd, Model MP 100 1759, Bangkok) connected through data acquisition equipment to a computer. There were 4 channels helping in simultaneous recording of 4 aorta rings. The experimental set-up is shown in figure 1.



Figure 1: Set up for isolated organ bath. [Heto Lab Equipment BIOPAC System (water bath), Scientific Promotion Co. Ltd., Model MP 100 1759 Soiwachirathumsathit, 11 Sukhumvit, 101/1 Bangkok, Prakanong, Bangkok 10260; Programme – Labscribe 2 – iWOR x 1, Washington Street, DOVER NH 03820]

A series of experiments were conducted with different drugs and different concentrations of noradrenalin (NA) to standardize our protocols.

We selected only two drugs namely *Aurum Metallicum 30 CH* and *Lycopus V 30 CH*. Sealed vials of the drugs, manufactured by Reckeweg, Germany, were purchased from the market in Kolkata. Potentized homeopathic drugs are normally produced from their mother tinctures through successive dilution with 90% ethanol 1:100 followed by mechanical agitation or succussion in as many steps as the potency number. Thus for a 30 CH potency, used in the present study, the drugs were prepared in 30 steps of serial dilution followed by succussion [1].

A basal tension of 1g was applied to the vascular rings. After a 90 min period of incubation 10  $\mu$ l of NE at a concentration of  $10^{-7}$  M was added to the bathing solution in the organ bath. An initial rise in tension occurred, followed by a plateau which was maintained for 30 min. In the control the diluent medium of 90% ethanol was added at 15  $\mu$ l within 10 min of NA addition. For the tests, the same amount of drugs *Aurum met 30 CH* or *Lycopus V 30 CH* was added to separate baths. The bathing solution was replaced by fresh solution every 20 min in the organ baths. After each experiment the tissues were washed 5 times with the bathing solution. Each experiment with a drug or the control solution was repeated 6 times.

## Results

The drugs produced marked relaxation of NA-induced contraction of aorta. The data are expressed in terms of percent inhibition of NA induced contraction [11]. Percent inhibition with two drugs used with their standard errors are presented in histograms in figure 2. *Aurum met 30 CH* and *Lycopus V 30 CH* produced 93.3 % and 90.8 % inhibition of NA induced contraction of rat aorta, respectively.

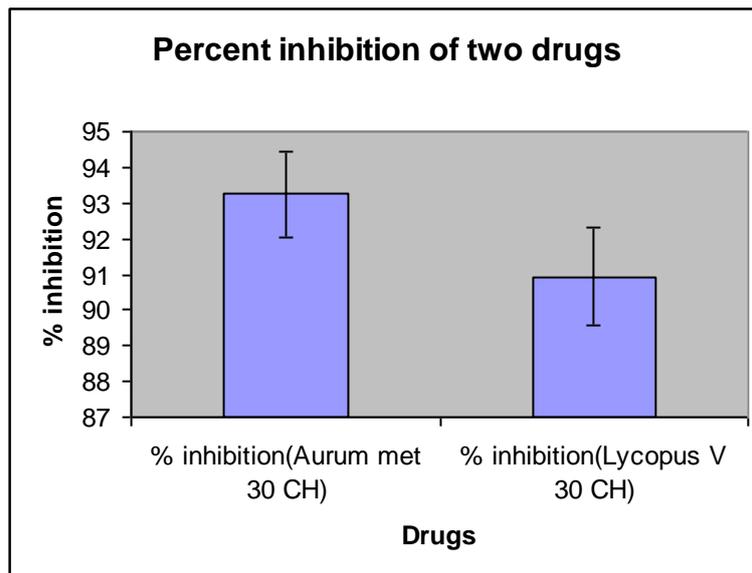


Figure 2: Percent inhibition of NA induced contraction of rat aorta by two homeopathic drugs.

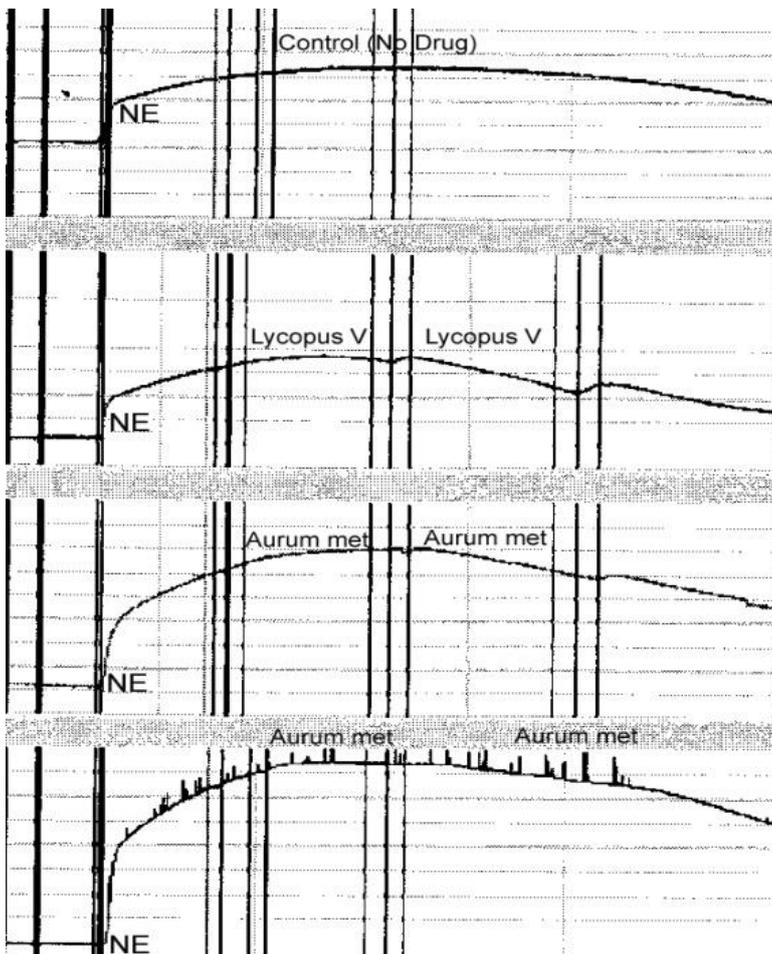
A comparison between actual NA induced contraction and drug induced relaxation has been made in Table 1. Significant difference from NA induced contraction after addition of drugs occurs ( $P < 0.001$  by student t-test,  $n = 6$ ). The period between addition of drugs and appearance of response was 2.7 to 7.2 minutes for *Aurum met 30 CH* and 3.4 to 8.1 minutes in case of *Lycopus V 30 CH*. While one dose was sufficient to elicit the response in most cases, 2-3 doses at an interval of 2 minutes were necessary in some cases (table 2).

Table 1: Drug induced change in tension (gm) of rat aorta precontracted with noradrenalin (NA)

Drug / Control	Basal Tension (gm.)	Increase in Tension after NA	Net change in Tension after Drug
<i>Aurum Met</i> 30 CH	1	1.6 ± 0.31	0.18 ± 0.03
<i>Lycopus V</i> 30	1	1.82 ± 0.34	0.25 ± 0.04
Control	1	1.73 ± 0.26	1.55 ± 0.25

Table 2: Degree of response of Homeopathic potencies to the four concentrations of noradrenalin (NA) applied.

Concentration of NA applied	Contraction	Relaxation of aortic rings with	
		<i>Lycopus V</i> 30 CH	<i>Aurum met</i> 30 CH
10 <sup>-5</sup>	+++	Nil	Nil
10 <sup>-6</sup>	+++	+	+
10 <sup>-7</sup>	+++	+++	+++
10 <sup>-8</sup>	+	++	++



**Figure 3:** A representative sample showing actual changes in rat aorta rings after application of noradrenalin (NA) at 10<sup>-7</sup> M followed by 90 % ethanol (control), *Lycopus V* 30 CH, *Aurum met* 30 CH in four separate organ baths. The amount of NA, control or drug solutions added twice at an interval of 5min. Both the drugs produced relaxation of the aorta. Control (no drug) means 90 % ethanol only. The vertical lines which appear in the computer printing have no link with the data.

A representative sample showing the actual effect of the two drugs, *Aurum met 30 CH* and *Lycopus V 30 CH* on the NA induced contraction of rat aorta is shown in Fig. 3.

The concentration of NA is important and it should not exceed  $10^{-7}$  M (figure 4).

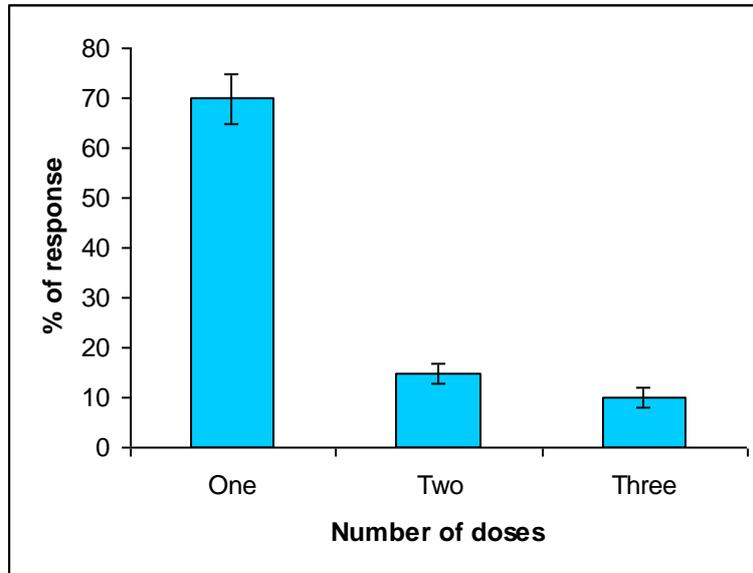


Fig. 4: Percentage of relaxation response of rat aortic rings in response to number of doses of homeopathic potencies applied.

## Discussion

The results provide evidence that the two drugs *Aurum met* and *Lycopus V*, which are known to be effective in reducing blood pressure in patients, show *ex vivo* effect in producing relaxation in rat aorta. These two potentized drugs do not contain any original drug molecules because their actual dilution at the 30<sup>th</sup> potency used in this study reaches  $10^{-60}$ .

The precise mechanism by which potentized *Aurum met* and *Lycopus V* act on the isolated aorta is not clear. Noradrenalin produces an excitatory effect on the force of contraction of heart and arteries via an action on adrenergic receptors [12, 13]. Sodium pump maintains tone and contractility of smooth muscles as in aorta [14]. Contraction and relaxation of isolated arteries depend on the combination of drug molecules with receptors in the vascular segment. Density and sensitivity of receptors are also important factors in this respect [11, 15]. Since homeopathic potencies used here do not contain any original drug molecules, the question of binding of drug molecules to smooth muscle receptors does not arise. Homeopathic potency-induced relaxation of aortic rings may involve non-receptor mechanism. One such mechanism involves prostaglandins released from endothelium [11]. But this possibility is ruled out because in the present study endothelium-denuded aortic rings were used. Mammalian tissues including the smooth muscle cells of rat aorta contain substantial amounts of taurine, an amino acid like compound which reduces hypertension [16]. The two homeopathic drugs tested might have stimulated release of endogenous taurine from the muscle cells thereby producing relaxation of aorta.

Homeopathic potencies are thought to comprise specific water structures [17, 18, 19]. These structures are preserved by ethanol, major component of a potency. While H-bonded three dimensional water structures represent the identity of a potentized drug, the H-bond strength carries the information of its potency [1, 20]. Water serves as a carrier of information of drug effect from one plant to another [21]

If this is so then the potencies may act on the water structure of the bathing fluid which is in contact with the smooth muscle of aorta. The changed water structure in the bathing solution may influence the relaxation of the aorta. It has been observed that the homeopathic potencies used in this study, do not elicit any response in the rat aorta unless the vascular rings are pre-contracted. This shows that in order to get response with homeopathic drugs a disease condition has to be produced. In this case blood pressure or vascular contraction was produced with NA so that anti-hypertension drugs like *Aurum met* and *Lycopus V* could produce their effect on the isolated aorta. We have further observed that maintaining appropriate muscle tone is necessary to elicit response with homeopathic potencies. The smooth muscles in isolated arteries show fatigue with the lapse of time resulting in reduced tension in a relatively shorter time [22]. All these factors should be taken into account to get reproducible results.

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## **Altas diluições de medicamentos homeopáticos induzem relaxação da aorta de rato, contraída mediante noradrenalina**

### **RESUMO**

**Introdução e objetivo:** Segundo alguns estudos, diluições homeopáticas alteram a contratilidade de íleo isolado de rato, em banho de órgãos. Os medicamentos homeopáticos como *Aurum metallicum* *Lycopus virginicus* são usados no tratamento da hipertensão. O objetivo deste estudo foi determinar se *Lycps* 30 cH e *Aur* 30 cH podem induzir o relaxamento da aorta isolada de ratos, em banho de órgãos. **Métodos:** A aorta foi dissecada e, livre de tecido conjuntivo e

endotelio, foi colocada em solução de Krebs-Henseleit, sendo seccionada em anéis de 2 a 2,5 cm de comprimento. Os anéis foram fixados em banho de órgãos, a extremidade superior ligada por um cabo a um transdutor isométrico, por sua vez ligado a dispositivos de registro de dados em um computador. Aur 30 cH, Lycps 30 cH e etanol 90%, foram adicionados separadamente ao líquido do banho contendo os anéis de aorta previamente contraídas pela noradrenalina (NA) ou norpinefrina (NE). **Resultados:** Os dois medicamentos testados induziram relaxamento significativo ( $p < 0,001$ ), do preparada de aorta contraída por NA ( $10^{-7}$  M), enquanto que a solução de controle não produziu efeitos significativos. **Conclusão:** Ambas as drogas foram usadas em diluições homeopáticas demasiado elevadas para admitir a presença de moléculas da substância original. Assim, o mecanismo farmacológico tradicional envolvendo as moléculas da droga e receptores no músculo liso da aorta deve ser descartado. Embora reconhecidamente diferente, o mecanismo envolvido permanece desconhecido. Lycps 30 e Aur 30 CH mostraram-se eficazes na redução da contratilidade da aorta de ratos, induzida por NA. As drogas podem agir diretamente na aorta isolada de ratos sem influência sistêmica.

**Palavras-chave:** altas diluições aorta isolada, hipertensão, noradrenalina.

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## Altas diluciones de medicamentos homeopáticos inducen relajación de aorta de rata contraída mediante noradrenalina

### RESUMEN

**Introducción y objetivos:** Según algunos estudios, diluciones homeopáticas alteran la contractilidad de íleo aislado de rata en baño de órganos. Medicamentos homeopáticos como *Lycopus virginicus* y *Aurum metallicum* son utilizados en el tratamiento de la hipertensión arterial. El objetivo de este estudio fue determinar si *Lycps* 30 cH y *Aur* 30 cH pueden inducir relajación de aorta aislada de rata en baño de órganos. **Métodos:** La aorta fue disecada y, libre de tejido conectivo y endotelio, colocada en solución de Krebs-Henseleit, siendo seccionada en anillos de 2 a 2.5 cm de longitud. Los anillos fueron fijados en baño de órganos, estando el extremo superior sujeto por una cuerda a un transdutor isométrico, a su vez conectado por aparatos para registro de datos a una computadora. *Aur* 30 cH, *Lycps* 30 cH y su medio, etanol al 90%, fueron separadamente añadidos al líquido del baño conteniendo los anillos de aorta previamente contraídos mediante noradrenalina (NA) o norpinefrina (NE). **Resultados:** Los dos medicamentos homeopáticos testeados indujeron relajación significativa ( $p < 0.001$ ) del preparado de aorta contraída mediante NA ( $10^{-7}$  M), mientras que la solución de control no produjo efectos notables. **Conclusiones:** Ambos medicamentos homeopáticos fueron utilizados en diluciones demasiado altas como para admitir la presencia de moléculas de la sustancia original. De este modo, el mecanismo farmacológico tradicional implicando moléculas de droga y receptores en el músculo liso de la aorta debe ser descartado. A pesar de reconocidamente diferente, el mecanismo implicado permanece desconocido. *Lycps* 30 cH y *Aur* 30 cH se mostraron efectivos en la reducción de la contractilidad de aorta de rata inducida por NA. Los fármacos pueden actuar directamente sobre aorta aislada de rata sin ejercer influencia sistémica.

**Palabras clave:** Altas diluciones, aorta aislada, hipertensión arterial, noradrenalina.



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