

# The problem of dose in homeopathy: evaluation of the effect of high dilutions of *Arsenicum album* 30cH on rats intoxicated with arsenic

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## ABSTRACT

**Background:** Although scientific studies have confirmed the action of homeopathic high dilutions in living organisms an endless debate on the choice of the most fitting dilution, the frequency of administration and the dose (amount of medicine) still remains. **Aims:** This study sought to assess the *in vivo* effect of 2 different concentrations of *Arsenicum album* 30cH in order to elucidate some problems in the homeopathic notion of dose. **Methods:** Male Wistar rats previously intoxicated with sodium arsenate by peritoneal injection were treated with undiluted *Ars* 30cH and *Ars* 30cH in 1% solution administered by oral route. Atomic absorption spectroscopy was employed to measure the levels of arsenic retained in the animals as well as the amounts eliminated through urine. Urine samples were collected before and after and during treatment. A positive control group (intoxicated animals) and negative control group (non-intoxicated animals) were administered only the vehicle used to prepare the medicine (ethanol). **Results:** The groups treated with undiluted *Ars* 30cH and *Ars* 30cH in 1% solution eliminated significant amounts of arsenic through urine when compared to the control groups. The group treated with undiluted *Ars* 30cH eliminated significantly higher amounts of arsenic than the group treated with the same medicine in 1% solution. **Conclusion:** These results suggest that undiluted *Ars* 30cH was more effective than in 1% solution in this experimental model.

**Keywords:** Homeopathic medicines; Dose; Experimental model; Rats; Arsenic intoxication; *Arsenicum album*

## Introduction

Although scientific studies have confirmed the action of homeopathic high dilutions in living organisms [1-15], many doubts remain over their practical application. Aside from an endless debate on the choice of the most fitting dilution and the frequency of administration of homeopathic medicines to actual patients, there is also an ongoing and heated discussion on whether the dose (amount of medicine) is also a relevant factor [16-22].

In conventional medicine, the issue of doses (amounts of medicine) is relatively simple, since conventional pharmacology is essentially based on the quantitative relation among the taken dose, the concentration of the drug in the body and the magnitude of the effect (dose-response curve) [23]. Conversely, it is a much more complex matter in homeopathy largely due to the inability to measure the amount of drug contained in the medicines. For this reason, different opinions have been raised throughout the history of homeopathy [24].

According to some authors, the pharmacological concept of dose, namely the amount of drug a patient must take to elicit a change in his or her state of health does not apply in homeopathy. The reason adduced is that homeopathic medicines act through “dynamic” or “qualitative” rather than mass action, whereas the duration of its effect depends on the power of reaction or the “sensitivity” of the ill body [25-28]. Conversely, other authors invoking their personal experience have emphasized the importance of doses in the therapeutic outcomes [29, 30].

A study carried out in 2001 by the Brazilian Association of Homeopathic Pharmacists showed that most pharmacies surveyed in the State of São Paulo dispense homeopathic medicines in 1% solution, for no specific reasons [31].

The aim of the present study was to evaluate in an *in vivo* experimental model whether there are differences in the effects of homeopathic medicines when prescribed with or without further dilution, i.e. in a larger or smaller dose (amount of medicine) of the same homeopathic preparation. The model chosen was homeopathic medicine *Arsenicum album* in experimental arsenic intoxication in rats [32-37].

## Material and Methods

### *Preparation of the homeopathic medicines*

*Arsenicum album* 30cH was prepared in 30% ethanol in 2 samples: 1) Hahnemann's trituration method for the 3 first solutions, starting from arsenic trioxide, followed by Hahnemann's method of multiple flasks for the next dilutions; and 2) 1% solution of Ars 30cH (1 part of Ars 30cH in 99 parts of 30% ethanol), as described in the 2<sup>nd</sup> edition of Manual of Technical Guideline by the Brazilian Association of Homeopathic Pharmacists [38].

### *Intoxication of animals*

Male Wistar rats were divided into 4 groups of 5 animals each; animals were purchased from ANILAB, Paulínia, São Paulo, Brazil.

Animals in groups 1, 2 and 3 (G1, G2 and G3 respectively) were intoxicated with 70 mg of sodium arsenate corresponding to 16,8 mg of arsenic/kg, as established in a previous study [39]. Animals in group 4 (G4) were not intoxicated. Arsenic was administered in solution by intraperitoneal injection. The animals were kept in individual metabolic cages with *ad libitum* water, treated with balanced, CR-1 diet (Nuvital) and submitted to cycle of 12h light/dark under controlled temperature (23±2 °C) throughout the study.

This study followed the procedures described in Brazilian government Resolution n° 714 (20/06/2002) and Law n° 11794 (11/10/2008).

### *Collection of urine samples*

Urine samples were collected in 20 ml-volume amber-colored glass flasks previously cleaned, dried and identified. The flasks were placed below the metabolic cages and covered at all times with a piece of gauze to avoid the mixing of rests of ration and urine. Urine samples were collected during 24 hours before

experimental intoxication (BI) to verify the presence of eventual elimination of arsenic. Further samples were collected during 48 hours after intoxication before the onset of treatment (T0) and during days 6 and 7 (T6), 14 and 15 (T14) and 33 and 34 (T30) after the onset of treatment.

### ***Treatment of animals***

Animals in G1 received p.o. 0.1 ml of *Ars* 30cH and in G2, 0.1 ml of 1% *Ars* 30cH, once a day. Animals in G3 (positive control) and G4 (negative control) received p.o. 0.1 ml 30% ethanol, once a day, as placebo. Medicines and placebo were administered on days 2, 3 and 4 after intoxication, then again after a 3-day interval on days 8, 9 and 10, and finally after a second 3-day interval, on days 22, 23 and 24 after intoxication.

### ***Quantification of arsenic***

Urine samples collected from each animal were filtered in qualitative filter paper and kept at 10°C until they were subjected to acid digestion with sulphuric acid and heat [40] as follows: urine samples were transferred to glass tubes 25 cm high and 2.1 cm diameter. The tubes were carefully placed in a Tecnal Block Digester, model TE-040/25 kept at about 350°C. Sulphuric acid was carefully added to the urine samples until a clear and transparent solution was obtained. After acid digestion was completed, the urine samples were placed in 20 ml amber-colored flasks previously cleaned, dried and identified.

In order to determine the concentration of arsenic in the bone and cartilaginous tissues, 2 rats in each group were randomly chosen; animals in each group were identified by numbers and chosen by lottery. After sacrificed with carbon dioxide gas, their back legs were dissected, the excess of muscular and fat tissue in each leg was removed in order to obtain bone and cartilaginous tissue; the latter were weighed and kept in a stove at 50°C until weight stabilized. Then, the bone and cartilaginous materials were fragmented into a fine powder and subjected to acid digestion as described above. To quantify the arsenic (As) eliminated by urine or present in the bone and cartilaginous tissue, 500 µl of the samples were diluted in a 0.5% solution of ascorbic acid and potassium iodide to allow for the reduction of  $As^{5+}$  into  $As^{3+}$ . Then, concentrated HCl was added until reaching 30% (v/v) necessary for the maintenance of the flame in the detector. The determination of arsenic was carried out through the generation of hydrates, by reducing the solutions of arsenic with 1.3% solution of  $NaBH_4$  in NaOH. The measurement of the concentration of arsenic was performed in duplicate by atomic absorption spectroscopy (PS Analytical) using an Excalibur detector. The presence of arsenic in sulphuric acid, the ration and purified water used during the study was also investigated.

### ***Statistical Planning and Analysis***

The results obtained in each group at the different times of the study were analyzed through Kruskal Wallis Zar test [41]. Comparisons between groups were made through Kolmogorov-Sminov Z test and significance level 0.05 was established for 2 groups [42]. Analyses were carried out with software SPSS 13.0.

### **Results**

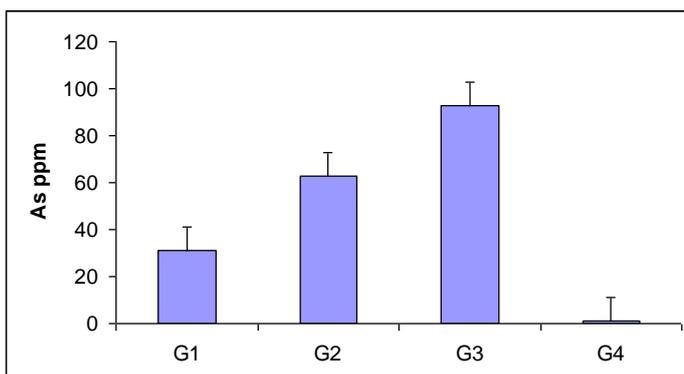
Non-significant amounts of arsenic were found in the ration (1.05 ppm), sulfuric acid (1.29 ppm) and the urine of animals before intoxication (Table 1). Arsenic was not detected in the purified water used. Table 1 describes the median values and interquartile range of arsenic eliminated at different times.

Figure 1 shows the average amount of arsenic found in the bones and cartilage of animals in G1, G2, G3 and G4 at the end of the experiment, which respectively were 31.31; 62.79; 93.02 and 1.062 ppm.

**Table 1** – Concentration of arsenic (ppm) eliminated in urine before intoxication of animals with arsenic (BI), 48 hours after intoxication and before treatment (T0), and on days 6 and 7 (T6); 14 and 15 (T14) and 33 and 34 (T30) after treatment (n = 5).

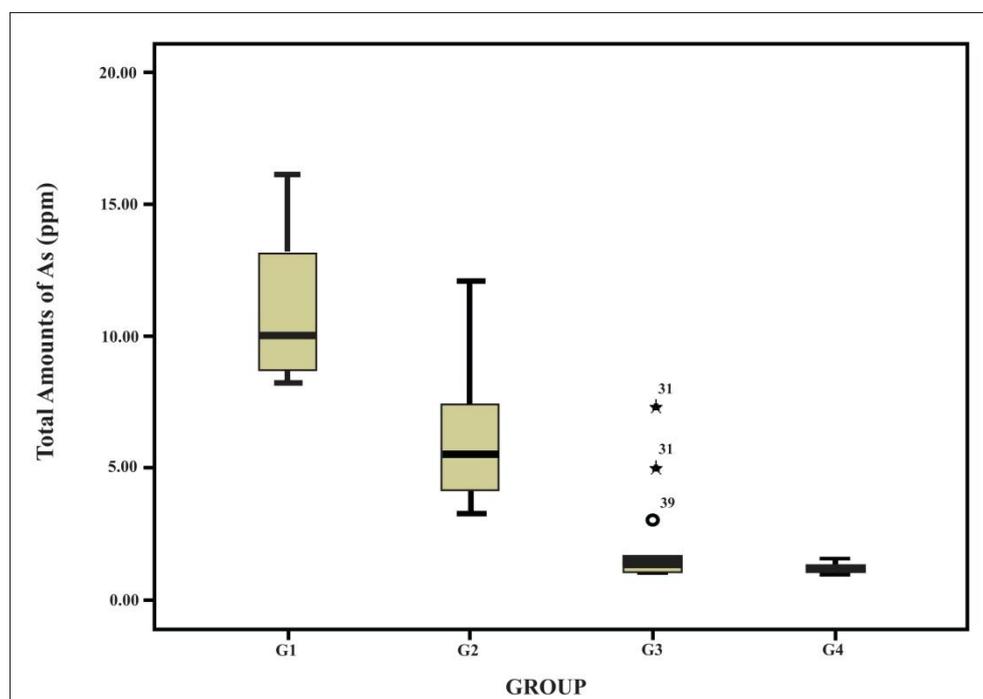
| Period     | Group |                  | Median                          | % VAR* | % VAR* |
|------------|-------|------------------|---------------------------------|--------|--------|
|            |       |                  | (Percentile 75 – Percentile 25) | G3     | G4     |
| <b>BI</b>  | G1    | Untreated        | 0.00 (0.00)                     | 0%     | 0%     |
|            | G2    | Untreated        | 0.00 (0.00)                     | 0%     | 0%     |
|            | G3    | Positive control | 1.16 (0.17)                     |        |        |
|            | G4    | Negative control | 1.02 (0.06)                     |        |        |
| <b>T0</b>  | G1    | Untreated        | 0.03 (0.00)                     | -33%   | -17%   |
|            | G2    | Untreated        | 0.03 (0.01)                     | -13%   | 8%     |
|            | G3    | Positive control | 0.04 (0.04)                     |        |        |
|            | G4    | Negative control | 0.03 (0.02)                     |        |        |
| <b>T6</b>  | G1    | Treated          | 11.56 (4.32) <sup>abc</sup>     | 634%   | 844%   |
|            | G2    | Treated          | 6.29 (3.03) <sup>ae</sup>       | 310%   | 414%   |
|            | G3    | Positive control | 1.53 (1.17) <sup>bd</sup>       |        |        |
|            | G4    | Negative control | 1.22 (0.18) <sup>ce</sup>       |        |        |
| <b>T14</b> | G1    | Treated          | 12.38 (3.20) <sup>abc</sup>     | 642%   | 1091%  |
|            | G2    | Treated          | 5.30 (3.28) <sup>ae</sup>       | 218%   | 410%   |
|            | G3    | Positive control | 1.67 (2.02) <sup>bd</sup>       |        |        |
|            | G4    | Negative control | 1.04 (0.07) <sup>ce</sup>       |        |        |
| <b>T30</b> | G1    | Treated          | 9.31 (1.14) <sup>ade</sup>      | 575%   | 668%   |
|            | G2    | Treated          | 5.48 (2.55) <sup>bc</sup>       | 297%   | 352%   |
|            | G3    | Positive control | 1.38 (0.32) <sup>bd</sup>       |        |        |
|            | G4    | Negative control | 1.21 (0.45) <sup>ce</sup>       |        |        |

Median values followed by equal small letters indicate significant difference between the groups by *Kolmogorov-Smirnov* Test ( $p < 0.05$ ) \*%VAR: represents percentage of variation regarding the average. G1 and G2: groups treated with undiluted *Ars* 30cH and 1% *Ars* 30cH respectively; G3: positive control; G4 negative control.



**Figure 1** – Concentration of arsenic (ppm) retained in the cartilage and bones of intoxicated animals treated with undiluted *Ars* 30cH (G1), 1% *Ars* 30cH (G2), 30% ethanol (G3) and not intoxicated (G4) (n = 6, bars represent SD).

Figure 2 shows the comparison between all 4 groups as to the total amount of arsenic (ppm) excreted in urine during the study.



**Figure 2** – Comparison between the total amount of arsenic (ppm) eliminated by urine after the onset of treatment ( $p = 0,01$ ). G1 and G2: groups treated with undiluted *Ars* 30cH and 1% *Ars* 30cH; G3: positive control; G4: negative control ( $n = 6$ , bars represent SD).

## Discussion

This study is the follow-up of a previous one [31] designed in order to further the understanding of the notion of dose in homeopathy. This model showed the efficacy of high dilutions of *Arsenicum album* to promote the elimination of arsenic retained in the body [32-37].

In the present study, animals in G1 (treated with undiluted *Ars* 30cH) eliminated significantly greater amounts of arsenic through urine compared to the control groups (G3 and G4) at times T6, T14 and T30, showing its efficacy in stimulating the urinary excretion of arsenic in experimentally intoxicated rats. Compared to G2, the results of G1 were statistically higher ( $p < 0.05$ ) at times T6 and T14 but not at T30.

The excretion of arsenic through urine in G2 (treated with 1% *Ars* 30cH) was significantly higher than in G4 at times T6, T14 and T30. No statistical difference with G3 was found at T6 or T14, but it was at T30. No difference was found between G3 and G4 in any of the 3 times analyzed. These findings suggest that besides quantitatively lesser, the action of the diluted medicine may have also been slower than the action of the undiluted one. The diluted medicine induced a significant level of excretion (by comparison to controls) only 30 days after the onset of treatment against 6 days with the undiluted medicine.

Regarding the ability to mobilize arsenic retained in bones and cartilage, G1 showed significantly higher results than G3. This finding corroborates the results of the elimination of arsenic in urine (Table 1). However, this was not seen when comparing G2 and G3 despite the difference in the elimination of urine at

T30. These results seem to corroborate the differences in urinary elimination of arsenic between G1 and G2 already mentioned.

In G1, the elimination of arsenic through urine was high at T6, increased at T14 and decreased at T30. This pattern was accompanied by lower amounts of arsenic in the bones and cartilage at the end of the study. Urinary elimination of arsenic was also observed in G2, albeit in lower amounts and at a constant rate across all times analyzed.

These data allow us to suggest that the effect of 1% *Ars 30cH* on the elimination of arsenic from the body of the animals was smaller than with the undiluted medicine (given the lower amount of arsenic detected in urine) or possibly that elimination was slower and did not attain its maximum during the period of study. Under ideal conditions, the exact amount of arsenic in the bones and cartilage ought to be measured after intoxication and before treatment, but this requires sacrificing the animals which makes further study impossible.

The results shown in Table 1 for G1 and G2 at BI and T0, and for G3 and G4 at BI, T0, T6, T14 and T30, as well as for G4 in Figure 1 fall within the limit of error of the method of analysis used and thus can be dismissed, since these values are similar to the concentrations of arsenic measured in the blank solution (sulfuric acid).

Figure 2 shows the comparison among all 4 groups based on the total amount of arsenic (ppm) excreted in urine throughout the study. The results depicted in Figure 2 reveal statistically significant difference for G1 compared to G2, G3 and G4 ( $p = 0.0001$ ). G2 data showed significant difference compared to G3 and G4 ( $p = 0.0002$ ). No statistically significant difference was found between G3 and G4 ( $p = 0.125$ ).

Our results indicate that the amount of medicine taken by the animals influenced the responses observed, given that there was statistically significant difference in the urinary excretion of arsenic between G1 and G2. A similar influence of the amount of homeopathic medicine was also observed in a previous study [31] using *Arsenicum album 6cH* in 1% solution.

Based on these findings, it can be concluded that the amount of medicine has an influence on the action of homeopathic medicines in living beings, and that this influence is independent from the homeopathic dilution used, since the same effect was observed both with high and low dilutions (viz. above and below Avogadro's number). In the actual clinical situation, however, other factors may also play a role, as e.g. the patient's sensitivity and capacity of reaction, as well as the interval between doses, etc., in the selection of the best prescription according to the requirement of individualization of homeopathic therapeutics.

## Conclusion

The present study confirmed the effect of homeopathic medicine *Arsenicum album 30cH* on the mobilization of semi-metal arsenic (As) previously stored in the body of rats. It proved effective to promote the elimination of arsenic when used both diluted (1%) and not diluted. The undiluted sample elicited the elimination of a significantly larger amount of arsenic than its diluted equivalent, indicating that the amount of medicine triggered different responses in a same experimental model.

The results of the present study confirmed that the dose of homeopathic medicine has an effect on the detoxification of rats previously intoxicated with sodium arsenate. These results can contribute towards a scientific definition of the homeopathic concept of dose.

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## O problema da dose em homeopatia: avaliação do efeito de altas diluições de *Arsenicum album* 30cH em ratos intoxicados com arsênico.

### RESUMO

**Introdução:** embora estudos científicos têm confirmado a ação das altas diluições homeopáticas em organismos vivos, permanece o debate infundável acerca da escolha da diluição mais adequada, a frequência de administração e a dose (quantidade de medicamento). **Objetivos:** este estudo procurou avaliar o efeito *in vitro* de 2 concentrações diferentes de *Arsenicum album* 30cH a fim de elucidar alguns aspectos da noção homeopática de dose. **Métodos:** ratos Wistar machos previamente intoxicados com arseniato de sódio por injeção peritoneal foram tratados com *Ars* 30cH não diluído ou diluído 1% por via oral. Foi utilizada espectroscopia de absorção atômica para medir os níveis de arsênico retido no organismo e eliminado através da urina. As amostras de urina foram colhidas antes, após e durante o tratamento. Os grupos controle positivo (animais intoxicados) e negativo (animais não intoxicados) receberam apenas o veículo utilizado para preparar o medicamento (etanol). Resultados: os grupos tratados com *Ars* 30cH não diluído e diluído em solução 1% eliminaram quantidades significativamente maiores de arsênico através da urina por comparação aos grupos controle. O grupo tratado com *Ars* 30cH não diluído eliminou quantidades significativamente maiores de arsênico que o grupo tratado com o mesmo medicamento em solução 1%. **Conclusão:** esses resultados sugerem que *Ars* 30cH não diluído foi mais efetivo que a solução 1% do mesmo neste modelo experimental.

**Palavras-chave:** Medicamentos homeopáticos; Dose; Modelo experimental; Ratos; Intoxicação arsenical; *Arsenicum album*

## El problema de la dosis en homeopatía: evaluación del efecto de altas diluciones de *Arsenicum album* 30cH en ratones intoxicados con arsénico.

### RESUMEN

**Introducción:** aunque estudios científicos han confirmado la acción de las altas diluciones homeopáticas en organismos vivos, permanece sin resolver la discusión acerca de la elección de la dilución más adecuada, la frecuencia de administración y la dosis (cantidad de medicamento).

**Objetivos:** este estudio buscó evaluar el efecto in vitro de 2 concentraciones diferentes de *Arsenicum album* 30cH para dirimir algunos problemas en la noción homeopática de dosis.

**Métodos:** ratones Wistar macho previamente intoxicados con arseniato de sodio por inyección peritoneal fueron tratados con *Ars* 30cH sin diluir o diluido en solución 1% por vía oral. Fue utilizada espectroscopía de absorción atómica para medir los niveles de arsénico retenidos en el organismo y eliminados por orina. Las muestras de orina fueron recogidas antes, durante y después del tratamiento. Los grupos control positivo (animales intoxicados) y negativo (animales no intoxicados) recibieron exclusivamente el vehículo utilizado para preparar el medicamento (etanol). Resultados: los grupos tratados con *Ars* 30cH no diluido y diluido 1% eliminaron cantidades significativamente mayores de arsénico por orina que los controles. El grupo tratado con *Ars* 30cH no diluido eliminó cantidades significativamente mayores de arsénico por orina que el grupo tratado con el mismo medicamento diluido 1%. **Conclusión:** estos resultados sugieren que *Ars* 30cH no diluido fue más efectivo que cuando diluido 1% en este modelo experimental.

**Palabras-clave:** Medicamentos homeopáticos; Dosis; Modelo experimental; Ratones; Intoxicación arsenical; *Arsenicum album*

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