Evaluation of the effect of different concentrations of *Arsenicum album* 6cH on intoxicated rats

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ABSTRACT

Aims: Homeopaths diverge on the concept of dose, i.e. the amount of drug that a patient must take to alter his or her state of disease. In order to stimulate reflections on this concept, this study sought to evaluate *in vivo* the effect of different concentrations of *Arsenicum album* 6cH prepared according to homeopathic pharmacotechnics. **Methods:** male Wistar rats were intoxicated with arsenic and then treated with *Arsenicum album* 6cH and *Arsenicum album* 6cH diluted at 1%, administered orally. The amount of arsenic retained in the animals' organism and that eliminated by urine were measured through atomic absorption spectroscopy. Samples of urine were collected before and after intoxication and during treatment. The positive control group (intoxicated animals) and the negative control group (non-intoxicated animals) received only the vehicle used in the preparation of the medicine. **Results:** Groups treated with *Arsenicum album* 6cH and *Arsenicum album* 6cH diluted at 1% eliminated significant amounts of arsenic when compared to the control groups. The group treated with *Arsenicum album* 6cH eliminated significantly higher amounts of arsenic than the group treated with the diluted medicine at 1%. **Conclusion:** results suggest that *Arsenicum album* 6cH should not be diluted as not to compromise its effectiveness in the treatment of rats intoxicated with arsenic.

Keywords: high dilution - dosage in high dilutions - *Arsenicum album*.

Introduction

Homeopathy is a medical and pharmaceutical speciality officially recognized in Brazil by the federal councils of medicine and pharmacy. Its research methodology is supported by data from toxicology and clinical experimentation of drugs in healthy individuals [1]. The drug, however, must be turned into a high diluted medicine by pharmacists by means of a technique termed dynamization [2].

The technique of dynamization is regarded to avoid the aggravation of the symptoms and the toxic effects of the original substance when the high diluted medicine is administered and the reactive capacity of the live organisms is stimulated [3, 4].

The pharmacological activity of the high diluted medicines is reported in scientific journals. Among these works highlight those that use experimental models with high dilutions of endogenous molecules [5-8]; those that use models based on the prevention of intoxications using high dilutions [9-17] and those that use models based on the similitude principle [18-20].

According to some authors, the pharmacological concept of dosage, as the amount of medicine that a patient must take in order to modify the condition of his disease, cannot be applied to high diluted medicines, since the medicine does not act in virtue of its mass but rather by its qualitative dynamic effect [21-23]. Based on this interpretation of the homeopathic theory the degree of dynamization (potency) and the frequency of administration of the medicine, have been regarded as the most important factors in the choice of criteria for the prescription of the medicines. However, the size of the dosage to be used, mainly in acute episodes or in the case of very sensitive patients, has been the reason for frequent discussions that have not succeeded in bringing to an end the above mentioned controversy [24-29].

Several studies, using animal models, have shown the effect of the high diluted *Arsenicum album* on the elimination of the semi-metal arsenic previously retained by the organism [12-14, 17, 30-35]. However these studies did not evaluate the impact of the action of different dosages of this high diluted preparation.

The objective of the present work is to evaluate, *in vivo*, the effect of *Arsenicum album* 6cH and *Arsenicum album* 6cH diluted at 1%, on Wistar rats previously intoxicated with arsenic in order to contribute to the discussions on the concept of dosage in homeopathy.

Material and Methods

Preparation of the homeopathic medicine

The high dilution of *Arsenicum album* was prepared in the sixth of the centesimal scale (6cH) in ethanol at 30%, both in its concentrated and in its diluted at 1% forms, by the Hahnemanian trituration method for the first three potencies and by means of the Hahnemanian method of multiple flasks for the last three potencies. The medicines were prepared in liquid form for oral use as described in Farmacopéia Homeopática Brasileira 2nd Edition. These samples were obtained from the arsenic trioxide.

Intoxication of the animals

Male Wistar rats were divided into 4 groups of 5 animals. The 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 per kilogram of body weight. The animals in group 4 (G4) were not intoxicated. The arsenic was administered as a solution by intraperitoneal injection and the animals were kept in individual metabolic cages, with *ad libitum* water and ration, throughout the study. The sodium arsenate dosage was established in a previous work [17].

Collection of the urine sample

Urine samples were collected in amber coloured glass flasks of 20 ml previously cleaned, dried and identified. The flasks were positioned underneath the metabolic cages and covered at all times with a piece of gauze material to prevent rests of ration from mixing with the urine. The urine samples were collected during the 24 hours before the intoxication of the animals (AI) in order to verify the eventual elimination of arsenic from the organism of the rats; and during the 48 hours after the intoxication process (T0). Samples were also collected during the sixth and seventh days (T6), the fourteenth and fifteenth day (T14) and the thirty third and thirty fourth days (T30) after the beginning of the treatment.

Treatment of the animals

The medicines and the placebo were administered on the 2nd, 3rd and 4th days after the intoxication of the animals. They were again administered after a three day interval, during three consecutive days, in the 8th, 9th and 10th, that is on the 16th, 17th and 18th days; and, finally, after another three day interval, that is, on the 22nd, 23rd and 24th days after the intoxication of the animals. The animals in groups 1 and 2 were treated by oral administration of 0.1 ml of *Arsenicum album* 6cH and *Arsenicum album* 6cH diluted at 1%, respectively, once a day. The doses were measured with disposable syringes. The animals in groups 3 (positive control) and group 4 (negative control) were treated with 0.1 ml of ethanol at 30% (placebo).

Quantification of arsenic

Urine samples collected from each animal were filtered in qualitative filter paper and maintained at the temperature of 10°C until be submitted to acid digestion made with sulphuric acid and heat [36] as following: urine samples were transferred to glass tubes 25 cm high and with a diameter of 2.1 cm. The tubes were carefully placed in a Tecnal Block Digestor, model TE-040/25, kept at the approximate temperature of 350°C. Sulphuric acid was carefully added to the urine samples until a clear and transparent solution was obtained. After the acid digestion, the urine samples were placed in amber coloured flasks of 20 ml previously cleaned, dried and identified.

In order to determine the concentration of arsenic in the bone and cartilaginous tissues two rats of each group were randomly chosen. After they were sacrificed with carbon dioxide gas, their back legs were dissected. Then the excesses of muscular and fat tissue of each leg were removed in order to obtain the bone and cartilaginous tissues; the latter were weighed and kept in a stove at the temperature of 50°C until constant weight. After this procedure, the bone and cartilaginous material was fragmented to a fine powder and submitted to acid digestion, as described above. For quantification of arsenic (As) eliminated in urine or present in the bone and cartilaginous tissues, 500 µl [CRZ1] of the samples were diluted in a solution containing ascorbic acid and potassium iodide at 0.5% to allow the reduction from As⁵⁺ to As³⁺. Then, concentrated HCl was added until the proportion of 30% (v/v) was reached, which is necessary for the maintenance of the flame in the detector. The determination of the arsenic was carried out through the generation of hydrates, reducing the arsenic solutions with a NaBH₄ solution at 1.3% (w/v) in NaOH. Quantification of arsenic concentrations were performed in duplicate by atomic absorption spectroscopy (PS Analytical) using the Excalibur detector.

The presence of arsenic in the sulphuric acid, in the ration and in the purified water used at the different stages of the experiment was also investigated.

Statistical Planning and Analysis

The results obtained for the groups at the different times were analysed through the Kruskal Wallis Zar Test [37]. The groups were compared through the Kolmogorov-Sminov Z Test and the significance level of 0.05 was considered for two groups [38]. The analyses were carried out with the help of the software SPSS 13.0.

Results

Minor amounts of arsenic were found in the ration (1,02 ppm), in the sulphuric (1,29 ppm) acid and in the urine (Table 1) of the animals before their intoxication. Arsenic was not detected in the purified water also. Table 1 portrays the median values and the interquartile amplitude of the arsenic eliminated at the different times.

Figure 1 shows the average amount of arsenic found in the bones and cartilages of the animals in groups 1, 2, 3, and 4 at the end of the experiment which were, respectively, 68.02; 342.897; 273.122 and 1.258 ppm.

Figure 1 – Concentration of As (ppm) retained in the cartilages and bones of intoxicated animals and treated with *Arsenicum album* 6CH (G1), *Arsenicum album* 6CH diluted at 1% (G2) ethanol at 30%.

Table 1 – Concentration of arsenic (ppm), eliminated in urine before the intoxication of the animals (AI), 48 hours after the intoxication, before the treatment began (TO); during the 6th and 7th (T6); 14th e 15th (T14) and 33th e 34th (T30) days after the treatment (n = 5). Median values followed by equal small letters indicate a significant difference between the groups by the *Kolmogorov-Smirnov* Test (p<0,05) *%VAR: represent percentages of variation in relation to the average. G1 and G2: groups treated, respectively with *Arsenicum Album* 6CH and *Arsenicum Album* diluted 6CH at 1%.

Period	Group		Median (Percentile 75 – 25)	% VAR* G3	% VAR* G4
AI	G1	Untreated	1.08 (0.27)	-1%	20%
	G2	Untreated	1.29 (0.25)	-18%	6%
	G3	Positive Control	1.30 (0.28)		
	G4	Negative Control	1.39 (0.88)		
T0	G1	Untreated	1.02 (1.15)	43%	9%
	G2	Untreated	0.53 (0.53)	42%	-48%
	G3	Positive Control	1.28 (0.39)		
	G4	Negative Control	0.98 (1.09)		
T6	G1	Treated	12.10 (5.76) ^{abc}	1082%	1082%
	G2	Treated	3.50 (0.30) ^{ade}	-229%	-162%
	G3	Positive Control	1.12 (0.23) ^{bd}		
	G4	Negative Control	1.07 (0.11) ^{ce}		
T14	G1	Treated	28.07 (17.38) ^{abc}	1610%	1532%
	G2	Treated	3.17 (0.05) ^{ade}	-83%	-74%
	G3	Positive Control	1.89 (0.20) ^{bd}		
	G4	Negative Control	1.93 (0.77) ^{ce}		
T30	G1	Treated	21.79 (8.86) ^{abc}	-841%	1246%
	G2	Treated	6.08 (1.95) ^{ade}	-122%	-218%
	G3	Positive Control	2.55 (0.74) ^{bdf}		
	G4	Negative Control	1.86 (0.84) ^{cef}		

Figure 2 shows a comparison between the different groups (1, 2, 3 and 4) on the basis of the total amounts of arsenic (ppm) excreted in urine during the treatment.

Figure 2 – Comparison between the total amounts of As (ppm) eliminated in the urine after the beginning of the treatment ($p = 0,01$).

Discussion

G1 and G2 eliminated significant amounts of arsenic by comparison with the control groups (G3 and G4). However, G1 eliminated a significantly greater amount ($p < 0.05$) of arsenic than G2, G3 and G4 in T6, T14 and T30. The results of G2 showed to be significantly different from G3 and G4 at the times T6, T14 and T30, while the results of G3 showed statistically significant difference for G4 at T30.

The capacity of mobilization of the arsenic retained in the bones and cartilages was significantly greater in G1 than in G3 (positive control) corroborating the results found in the analysis of the arsenic eliminated in the urine (see Table 1). Nevertheless, G2 presented results statistically similar to G3 in bones and cartilages assays, although the elimination of arsenic in urine was significantly different between groups G2 and G1. Examining the results of elimination in urine for G1 it is possible to observe that the amount of arsenic eliminated is larger in T6, increases in T14 and tends to diminish in T30, while a reduction of the amount retained in the bones and cartilages at the end of the experiment is noted. However, in the case of G2, the amount eliminated in the urine is smaller than in G1, but tends to increase at all the times analyzed. Thus, we can suggest that the effect of the medicine administered in G2 (*Arsenicum album* 6cH diluted at 1%) was less pronounced in the elimination of the arsenic from the organism of the animals (as can be deduced by the

smaller amount found in the urine of this group) or the elimination may have been slower in this group by comparison with G1 (*Arsenicum album* 6cH) and did not reach the maximum arsenic mobilization in the time of the study. Since the elimination of the arsenic in G2 is progressively greater than in G3, the amount retained in the bones and cartilages would tend to decrease with time. The ideal situation would be the determination of the exact amount of arsenic retained in the bones and cartilages after the intoxication and before the beginning of the treatment. In order to know this, however, it would have been necessary to sacrifice the animals, and this would prevent us from proceeding with the experiment.

The results presented in Table 1 for groups 1 and 2 at AI and T0 and for the groups 3 and 4 at AI, T0, T6, T14 and T30 and in Figure 1 for G4 are within the margin of error of the methodology used in the analytical procedure and may be regarded as equal to zero, since these values are similar to the concentration of arsenic determined in the blanc (H₂SO₄).

The results presented in Figure 2 indicate a significant difference between group 1 and groups 2, 3, and 4 (p=0.013). For the data of group 2 there was statistically significant difference in relation to the data of groups 3 and 4 (p=0.013). No statistically significant difference was found between the results of groups 3 and 4 (p=0.819).

The results indicate that the amount of *Arsenicum album* taken by the animals interfered with the responses obtained, since groups G1 and G2 presented significant differences in the elimination of the arsenic within the same experimental model.

Conclusion

The present study confirmed the effect of the high diluted *Arsenicum album* 6cH on the mobilization of the semi-metal arsenic (As) previously retained in the organism of rats. Both in its concentrated and in its diluted form (at 1%), this medicine was effective in the arsenic elimination. However the concentrated medicine eliminated a significantly greater amount of arsenic than the diluted one, showing that the amount of medicine ingested led to different responses within the same experimental model. In the clinical practice, however, another relevant factors in the homeopathic therapeutic such as the patient's sensitivity and capacity of reaction as well as the frequency in the administration of doses should be considered so that an appropriate criterion of prescription may be established in accordance with the individualization of the homeopathic treatment. With this work we hope to contribute towards a scientific definition of the concept of dosage in homeopathy and high dilution models.

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Avaliação do efeito de diferentes concentrações de *Arsenicum album* 6cH em ratos intoxicados

RESUMO

Objetivos: Há divergência entre os homeopatas a respeito do conceito de dose, entendida como a quantidade de medicamento que o paciente deve ingerir para modificar o estado de doença. Para estimular a reflexão sobre este tópico, o presente estudo procurou avaliar o efeito *in vivo* de diferentes concentrações de *Arsenicum album* 6cH preparado segundo a farmacotécnica homeopática. **Métodos:** ratos Wistar machos foram intoxicados com arsênico e após, medicados com *Arsenicum album* 6cH e o mesmo diluído a 1% por via oral. O arsênico retido no organismo dos animais assim como o eliminado através da urina foi quantificado através de espectroscopia de absorção atômica. Amostras de urina foram colhidas antes e após a intoxicação e durante o tratamento. O grupo controle positivo (animais intoxicados) e o grupo controle negativo (animais não intoxicados) receberam apenas o veículo utilizado no preparo do medicamento. **Resultados:** os grupos tratados com *Arsenicum album* 6cH e *Arsenicum album* 6cH 1% eliminaram quantidades significativas de arsênico por comparação com os grupos controle. O grupo tratado com *Arsenicum album* 6cH eliminou quantidades significativamente maiores de arsênico que o grupo tratado com o mesmo medicamento diluído a 1%. **Conclusão:** os resultados sugerem que *Arsenicum album* 6cH não deve ser diluído a fim de não comprometer sua efetividade no tratamento de ratos intoxicados com arsênico.

Palavras chave: Altas diluições; Dose em altas diluições; Intoxicação; Arsênico; Ratos; *Arsenicum album*.

Evaluación del efecto de diferentes concentraciones de *Arsenicum album* 6cH en ratas intoxicadas

RESUMEN

Objetivos: Hay divergencias entre los homeópatas cuanto al concepto de dosis, entendido como cantidad de medicamento que un paciente debe ingerir para modificar su estado de enfermedad. Para estimular la reflexión sobre este tópico, este estudio buscó evaluar el efecto *in vivo* de diferentes concentraciones de *Arsenicum album* 6cH preparado según la farmacotécnica homeopática. **Métodos:** ratas Wistar macho fueron

intoxicadas con arsénico y a seguir, medicadas con *Arsenicum album* 6cH y *Arsenicum album* 6cH diluido al 1% por vía oral. La cantidad de arsénico retenida en el organismo así como la eliminada por orina fue medida mediante espectroscopía de absorción atómica. Muestras de orina fueron obtenidas antes y después de la intoxicación y durante el tratamiento. El grupo control positivo (animales intoxicados) e el grupo control negativo (animales no intoxicados) recibieron solamente el vehículo utilizado en la preparación del medicamento. **Resultados:** los grupos tratados con *Arsenicum album* 6cH y *Arsenicum album* 6cH 1% eliminaron cantidades significativas de arsénico en comparación con los grupos control. El grupo tratado con *Arsenicum album* 6cH eliminó cantidades significativamente mayores de arsénico que el grupo tratado con el mismo medicamento diluido al 1%. **Conclusión:** estos resultados sugieren que *Arsenicum album* 6cH no debe ser diluido para no comprometer su efectividad en el tratamiento de ratas intoxicadas con arsénico.

Palabras llave: Altas diluciones; Dosis de altas diluciones; Intoxicación; Arsénico; Ratas; *Arsenicum album*

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