Original Clinical Research 临床论著

An initial report on the efficacy of a millesimal potency Arsenicum Album LM 0/3 in ameliorating arsenic toxicity in humans living in a high-risk arsenic village

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Background: Millions of people are at risk of groundwater arsenic contamination, and there is no known remedy that can effectively remove the symptoms of prolonged arsenic poisoning. A potentized homeopathic drug, Arsenicum Album LM 0/3 (Ars Alb LM 0/3), is claimed in homeopathic literature to have the ability to treat symptoms similar to that of arsenic poisoning.

Objective: This study examines whether Ars Alb LM 0/3 could provide some degree of amelioration for the victims living in an arsenic-affected village where no arsenic-free drinking water is available.

Design, setting, participants and interventions: This study was carried out on volunteers living in an arsenic-affected village where no arsenic-free drinking water is available. Twenty-eight volunteers from the village of Dasdiya, in Haringhata block under Nadia District, West Bengal, India, an arsenic-contaminated village where wells contain 55 to 95 μg/L arsenic, were selected to undertake a double-blind and placebo-controlled trial. The subjects provided samples of blood and urine before and after 2 months of taking either "verum" or "placebo". Another 18 subjects living in an arsenic-free village, served as the negative controls.

Main outcome measures: Samples of blood and urine from the subjects were assayed for arsenic content, according to various toxicity biomarkers and pathophysiological parameters.

Results: Out of the original 28 subjects, only 14 subjects provided samples while the other

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14 dropped out. There were elevated levels of arsenic in the blood and urine, alkaline and acid phosphatases, lipid peroxidation, and glutathione activities and increased blood glucose, triacylglycerol, cholesterol, and low-density lipoprotein cholesterol contents, whereas there were decreased levels of aspartate and alanine aminotransferases, gamma glutamyl transferase, glucose-6-phosphate dehydrogenase contents, high-density lipoprotein cholesterol and packed cell volume in the subjects. After 2 months of homeopathic remedy administration, the verum-fed subjects showed positive modulations within these parameters with slight lowering of matrix metalloproteinase activity as compared with the placebo group.

Conclusion: Ars Alb LM 0/3 shows potential for use in high-risk arsenic villages as an interim treatment for amelioration of arsenic toxicity until more extensive medical treatment and facilities can be provided to the numerous victims of arsenic poisoning.

Keywords: arsenicals; homeopathy; arsenic poisoning; biological markers; matrix metalloproteinases; clinical trial

Contamination of drinking water with various toxic metal compounds, particularly arsenic, has become a menacing problem in approximately twenty countries so far. It affects millions of people, some 100 million alone in Bangladesh and parts of India (mainly in West Bengal)[1]. Reports of contamination in other areas are emerging, suggesting that this is going to be a global problem in the near future. Chronic exposure to arsenic affects the circulatory system and produces typical skin symptoms of arsenicosis, and can lead to various types of cancer in due course. So far, there is no proven orthodox medicine that can cure arsenicosis. Some chelating agents, such as dimercaptosuccinic acid and diethylenetriamine-pentaacetic acid, have been trialed but proven of little help[2]. Therefore, this study aims to identify agents which can combat chronic arsenic poisoning successfully, either by removing ingested arsenic from within the body or by combating the ill-effects produced by arsenic poisoning on different human systems, without being toxic themselves or having any adverse side effects, culminated in the use of a potentized homeopathic remedy, Arsenicum Album 30C (Ars Alb 30C)[3-5].

In earlier studies by the authors, it was reported that the homeopathic remedy Ars Alb 30C showed ameliorative potentials against chronic arsenic toxicity when administered for a short term[4] and even for a long period when shifted after some time to a higher potency Ars Alb 200C, that considerably ameliorated symptoms of arsenicosis both in respect of liver enzymes as well as skin symptoms[6]. Therefore, it is also necessary to reinvestigate the previous results by examining the efficacy of a fifty-millesimal or LM (as derived from Roman numerals: L=50, M=1 000) potency (LM 0/3) of Arsenicum Album in a small group of people living in an arsenic-contaminated village. As the LM potency is reported to give better results in repeatable doses over a longer period of time without the chances of any medicinal aggravation that sometimes preclude the long-term use of a centesimal potency of some homeopathic remedies[7].

1 Clinical data and methods

1.1 Clinical data

1.1.1 Study subjects This study was ethically approved by the Ethical Committee of the University of Kalyani, India, and with due permission from the Government of West Bengal, India. The trial was conducted under prescription and supervision of two qualified and licensed homeopathic practitioners as part of the team. The informed consent of the volunteers were also obtained prior to beginning this human trial. An awareness camp was organized in the village Dasdiya (which has recently been marked as an arsenic-contaminated village, arsenic contents of wells having measured between 55 and 95 μg/L) in Haringhata block under Nadia District, West Bengal, about the precautions and healthcare needed to combat chronic exposure to arsenic through groundwater contamination. The mission of testing the possible efficacy of a potentized homeopathic remedy, Arsenicum Album LM 0/3 (Ars Alb LM 0/3), was fully explained but most potential participants were reluctant due to a lack of confidence in homeopathy. On pursuance, 28 subjects, 12 males and 16 females showing initial signs or symptoms of arsenic poisoning agreed to act as volunteers for the double-blind placebo-controlled study only for 2 months.

1.1.2 Diagnostic criteria The subjects were medically assessed (for blood pressure, pulse rate, possible enlargement of spleen or liver, etc.) by two qualified homeopathic doctors in the team. They were also asked about their general physical condition such as appetite, bowel movements, sleep, urination, muscle or joint pain and skin itching. Subjects were questioned on a subjective basis, and whether any of these were, in their opinion, perceivably ameliorated after taking of the verum.

1.1.3 Inclusion criteria All the subjects, who were from the same socioeconomic background and had generally weak health, suffered from liver or alimentary system disorders, insomnia, complained of muscle or joint pain and those showing visible signs of arsenic toxicity such a burning sen-

sation of eyes and skin, rain drop pigmentation were included in the present study.

1.1.4 Exclusion criteria Subjects with noticeably poor state of health or with advanced cancer or terminal patients were excluded from this study.

1.2 Study methods

1.2.1 Study design Most arsenic victims were weak and anemic and therefore concerned about giving blood at regular intervals. They were in general frustrated and almost resigned to their fate. They signed informed consent on the basis of the agreement that they would provide samples of their urine and blood only twice; once on the day before they started taking the "verum" or "placebo", and then 2 months after administration of the "verum" or "placebo". Twenty-five similar bottles containing Ars Alb LM 0/3 and another 25 containing placebo (2% ethanol solution in which two medicine unsoaked sugar globules No. 10 were dissolved), marked with numerical codes (not disclosed to the researchers or the human volunteers), were kept on a tray. The subjects were asked to take any bottle randomly as per their choice. After 2 months, a sample-collection camp was held in the village to collect their urine and blood again.

However, only 14 subjects appeared at this time, 8 males and 6 females, who again gave their urine and blood samples for laboratory tests. On deciphering the codes it became apparent that of the 14 subjects who returned for retesting 9 had received the verum and 5 had received the placebo. Therefore, the sample size of the pilot study became rather small, only 9 Ars Alb LM 0/3-fed subjects, and 5 controls of placebo-fed were available. It was, therefore, decided to examine as many parameters of study as possible in order to arrive at an acceptable conclusion based on the totality of results. The data of all the 28 subjects who initially provided their blood samples were analyzed for different parameters vis-à-vis that of some 18 subjects, 14 males and 4 females, from a distant village Padumbasan in Midnapur (East) District, away from the Gangetic belt and known to be arsenic-free, to understand in the first place the impact of arsenic contamination in the various parameters of study. Furthermore, data from those volunteers who provided blood samples after 2 months of administration of homeopathic remedy or placebo were also analyzed for assessment of the ameliorative changes, if any, brought about by the remedy as compared with the placebo controls. In view of the small sample size, adequate statistical analyses were carried out to remove any "bias" in favor of or against the efficacy of the "verum" against "placebo", and the results thereafter presented. Figure 1 is the flow diagram of this trial.

1.2.2 Source, preparation and dose of the homeopathic remedy Ars Alb LM 0/3 was procured from Homeopathy International, Naity Road, Barabahera, Hooghly-712246, West Bengal, India. The preparation was made as per the millesimal potentization rules, diluting it according to the millesimal scale of 50 000[7]. Two tiny globules (No. 10) of the verum or placebo were dissolved in 100 mL of distilled water and mixed with 2 mL of absolute ethyl alcohol (i.e. 2% ethyl alcohol). The subjects were advised to give 10 up-and-down jerks to the bottle before taking 10 drops of the remedy twice daily, once on an empty stomach in the morning and once in the evening, at least an hour after or before taking any meal or food, and repeat in the similar way until the next collection of blood samples i.e. for 2 months. The placebo was produced in the same manner, except that only two unsoaked globules (No. 10) were put in 2% ethyl alcohol and were advised to be taken in the same procedure as that of the verum.

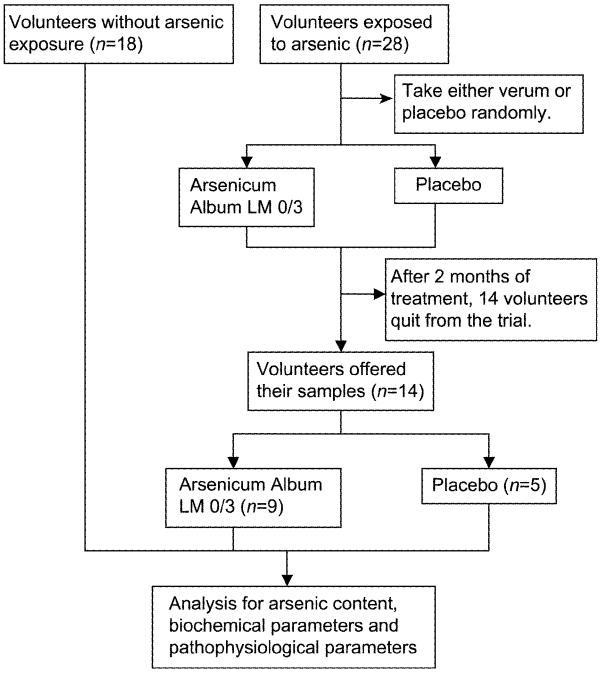

Figure 1 Flow diagram

1.3 Observed indexes

1.3.1 Collection of blood samples A sample of 6 mL of blood was drawn from each volunteer by venipuncture around the forearm region in the morning prior to intake of food by sterile disposable syringe and needle and put into two vials: one containing anticoagulant (ethylenediaminetetraacetic acid, EDTA) and the other without EDTA, and brought to the laboratory in a flask containing ice. Blood without EDTA was centrifuged at 2 000×g for 10 min and the serum was obtained.

1.3.2 Arsenic determination Arsenic content in urine and fasting blood of the volunteers of the first void morning was determined by a Perkin Elmer AAnalyst 200 Atomic Absorption Spectrophotometer (AAS) from USA adopting the standard protocol[8].

1.3.3 Biochemical studies Standard methods were used for the study of acid phosphatase (AcP), alkaline phosphatase (AlkP), aspartate

aminotransferase (AST), alanine aminotransferase (ALT), lipid peroxidase (LPO) and reduced glutathione (GSH)[9, 10] from blood serum. For the study of glucose-6-phosphate dehydrogenase (G6PD) activity, blood (uncoagulated) was subjected to diagnostic kit procured from Reckon Diagnostic Private Ltd., India. A sample of 0.1 mL of blood was washed with normal saline three times and erythrocytes were collected at the bottom of centrifuge tube treated with lysing reagent, and the enzyme substrate was added to it before it was read by a spectrophotometer at 340 nm. Similarly, for analysis of gamma glutamyl transferase (GGT), blood was subjected to the diagnostic kit supplied by Reckon Diagnostic Private Ltd., India.

1.3.4 Assay by standard kits and other standard methods For blood glucose determination, a standard glucose test kit (enzymatic, glucose oxidase-peroxidase method) obtained from Span Diagnostics Limited, India, was used and the level of blood glucose was determined by a spectrophotometer (Pharmaspec UV 1700, Shimadzu, Japan) at 550 nm. Hemoglobin content was determined by Sahli's method[11] with the help of a hemometer (Marienfield, Germany). For the determination of creatinine, the standard modified Jaffe's kinetic method[12] was followed. For determination of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerol, kits provided by Span diagnostics, India, were used. Blood with EDTA was used for determination of erythrocyte sedimentation rate (ESR), packed cell volume (PCV) and hemoglobin (Hb) by the standard laboratory methods. The procedures for media preparation and lymphocyte viability test have been described by Pathak *et al*[9] and Kwok *et al*[13].

1.3.5 Antinuclear antibody titer A small part of blood serum was taken for antinuclear antibody (ANA) test by using an ANA detect kit (ANA ORG 600; ORGENTEC Diagnostika GmbH, Germany) with the aid of an enzyme-linked immunosorbent assay (ELISA) reader (Eldex 3.8 ELISA Reader, USA; marketed in India by Lilac Medicare, Mumbai). This assay collectively detects, in one well, ANAs against some twenty antigens, including double stranded DNA (dsDNA, nDNA), histones, SS-A/Ro, SS-B/La, Sm, SmRNP, Scl-70, PM-Scl-100, Jo-1, and centromeric antigens. Blood sera of the ANA-positive samples were also subjected to the dsDNA, Scl-70 and alfa fetoprotein (AFP) antibody tests by the specific antibody kits (ANA ORG 600; ORGENTEC Diagnostika GmbH, Germany).

1.3.6 Gelatin zymography Gelatin zymography was used for determining the expressions of matrix metalloproteinases (MMPs) and methods described by Pathak *et al* were followed[9, 10].

1.4 Statistical analysis Data were expressed as $\overline{x} \pm s$. Significance of difference of scored data was compared by two-sample t test between negative control versus treatment groups and after verum administration versus after placebo administration groups. The paired t test was conducted to determine the significance level, if any, between before and after administration in each treatment group. A probability level of $P<0.05$ was considered statistically significant. Difference for significance by two-sample t test was determined by using Minitab15 Software, and the paired t test was conducted by the SPSS 10.0 software. Furthermore, as there were a large number of drop-outs, Bonferroni test was performed and multiple comparisons between these two treatments were conducted, so that the drop-out factor would not affect the analysis.

2 Results

2.1 Arsenic content in urine and blood Arsenic content in both urine and blood was found to be decreased in subjects taking either placebo or verum after 2 months, though the difference was not statistically significant when compared by paired t test (Table 1). Arsenic content in blood or urine in the control group residing in an arsenic-free village was below detectable limit. In both the placebo- and verum-fed groups of the arsenic village, the arsenic content in urine and blood was quite high. The initial difference noted in the arsenic content of placebo and verum groups could presumably be due to their actual difference of arsenic intake on the previous day, as arsenic is known to stay in blood for about 10 to 12 h before it is excreted through urine. However, in both the cases, the amount was much above the acceptable maximum level of arsenic content found in urine and blood of subjects living in arsenic-free control village[3, 5]. Since there was no arsenic-free water supply in this village, we could not conclude anything positive about the efficacy of the remedy in terms of the data of arsenic content in urine or blood.

2.2 Biochemical parameters As compared with the subjects living in the arsenic-free village, the subjects in Dasdiya had elevated levels of AcP, AlkP, and LPO and decreased levels of ALT, AST and G6PD. There were also a decrease of GGT and an increase of GSH levels. With regard to most parameters, the differences were statistically significant (Table 2). In the verum-fed group, there was a decrease in the activities of ACP, GGT and LPO as compared with the placebo-fed controls, and an increase of AlkP, ALT, GSH and G6PD activities and a marginal increase was noted in AST activity as well after 2 months (Table 3). The differences, when compared between placebo and verum after 2 months of administration, were mostly significant when analyzed by two-sample t test while others were non-significant (Table 3). However, when calculated by a paired t test between the before and after verum administration groups, most of the parameters showed

Table 1 Comparison of arsenic content in urine and blood samples of subjects fed with placebo or Arsenicum Album LM 0/3

($\bar{x}\pm s$, μg/mL)

Group	n	Arsenic content in urine		Arsenic content in blood	
		BP/BV	AP/AV	BP/BV	AP/AV
Placebo	5	53.97±2.57	50.72±11.50	12.40±5.33	7.39±4.71
Arsenicum Album LM 0/3	9	76.19±14.53	54.08±10.64	7.76±3.28	3.27±1.29

In two-sample t test analysis, when $P<0.05$, null hypothesis (H_0) was accepted i.e. differences were non-significant between AP and AV. In paired t test, when $P<0.05$, null hypothesis (H_0) was accepted i.e. differences were non-significant between BV and AV or BP and AP. BV: before verum; BP: before placebo; AV: after 2 months of verum; AP: after 2 months of placebo.

Table 2 Biochemical parameters in subjects before treatment against normal control

($\bar{x}\pm s$)

Group	n	AcP (nmol/(g protein·min))	AlkP (nmol/(g protein·min))	ALT (nmol/(g protein·min))	AST (nmol/(g protein·min))
Normal control	18	9.04±1.63	48.36±6.21	28.88±2.96	15.12±3.10
Study subjects before treatment	14	75.13±8.49**	98.30±5.18**	11.74±1.63**	6.01±1.29*
Group	**n**	**LPO (nmol MDA/mL sample)**	**GSH (nmol/mL sample)**	**GGT (IU/L)**	**G6PD (IU/L)**
Normal control	18	9.882±1.048	20.301±0.733	9.611±2.130	4.111±0.775
Study subjects before treatment	14	31.862±2.955**	21.809±0.768	9.546±3.224	1.001±0.416**

* $P<0.05$, ** $P<0.01$, *vs* normal control. AcP: acid phosphatase; AlkP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LPO: lipid peroxidase; MDA: malonaldehyde; GSH: reduced glutathione; GGT: gamma glutamyl transferase; G6PD: glucose-6-phosphate dehydrogenase.

Table 3 Biochemical parameters in subjects fed with placebo or Arsenicum Album LM 0/3

($\bar{x}\pm s$)

Group	n	AcP (nmol/(g protein·min))	AlkP (nmol/(g protein·min))	ALT (nmol (g protein·min))	AST (nmol/(g protein·min))
Placebo	5				
BP		88.4±21.2	107.8±14.0	12.1±3.7	7.9±3.2
AP		47.1±1.7	120.2±5.5	13.5±3.8	5.7±1.7
Arsenicum Album LM 0/3	9				
BV		67.7±6.1	92.9±1.7	11.5±1.6	4.9±0.9
AV		46.7±0.5▲	114.7±1.6▲▲	12.4±3.9	8.2±2.3
Group	**n**	**LPO (nmol MDA/mL sample)**	**GSH (nmol/mL sample)**	**GGT (IU/L)**	**G6PD (IU/L)**
Placebo	5				
BP		28.06±2.88	20.20±1.09	12.93±8.04	0.86±2.82
AP		5.02±1.29△△	23.98±0.85	3.85±0.57	2.82±0.66
Arsenicum Album LM 0/3	9				
BV		33.97±4.26	22.69±0.93	7.66±2.65	1.07±0.58
AV		6.17±0.41▲▲	25.83±0.89	5.22±0.66	2.11±0.51▲

In two-sample t test analysis, when $P<0.05$, null hypothesis (H_0) was accepted i.e. differences were non-significant between AP and AV. In paired t test, △△ $P<0.01$, *vs* BP; ▲ $P<0.05$, ▲▲ $P<0.01$, *vs* BV. BV: before verum; BP: before placebo; AV: after 2 months of verum; AP: after 2 months of placebo. AcP: acid phosphatase; AlkP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LPO: lipid peroxidase; MDA: malonaldehyde; GSH: reduced glutathione; GGT: gamma glutamyl transferase; G6PD: glucose-6-phosphate dehydrogenase.

statistically significant differences while there was little significant change in the placebo-fed group.

2.3 Pathophysiological parameters As compared with the subjects from the arsenic-free village, the subjects of the village Dasdiya had an increased level of blood glucose, triacylglycerol, creatinine, total cholesterol, LDL-C and ESR, and a decreased level of Hb content, PCV, HDL-C and the differences were statistically significant for most parameters when tested by two-sample t test (Table 4). There was a palpable decrease in the verum-fed subjects of all the parameters which were elevated as a consequence of arsenic exposure and an increase of hemoglobin content, PCV and HDL-C, as compared with the placebo controls. Some of the differences were statistically significant when compared by both two-sample and paired t tests (Table 5). However, the two-sample t test revealed only significant differences in respect of lymphocyte viability (Table 5). The viability of lymphocytes was less in subjects living in Dasdiya, as compared with that of Padumbasan (Table 4). The lymphocyte viability was increased in the verum-fed subjects, as compared with placebo-fed ones which was also statistically significant (Table 5).

Table 4 Pathophysiological parameters in subjects before treatment against normal control

($\bar{x}\pm s$)

Group	n	Blood glucose (mg/L)	Hb (g/L)	ESR (mm/h)	Total cholesterol (mg/L)	HDL-C (mg/L)
Normal control	18	902.4±34.8	101.0±2.1	5.00±0.89	1 815.3±134.1	656.7±113.4
Study subjects before treatment	14	925.5±85.7	75.0±6.3**	13.84±4.16*	1 837.0±163.2	301.1±44.8**

Group	n	LDL-C (mg/L)	Triacylglycerol (mg/L)	Creatinine (mg/L)	PCV (%)	Lymphocyte viability (%)
Normal control	18	1 050.5±270.5	1 498.0±102.3	6.2±0.5	43.55±1.00	92.44±3.90
Study subjects before treatment	14	1 262.4±133.4	1 533.6±180.5	11.5±2.4*	30.86±2.57**	73.99±1.49**

* $P<0.05$, ** $P<0.01$, *vs* normal control. Hb: hemoglobin; ESR: erythrocyte sedimentation rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PCV: packed cell volume.

Table 5 Pathophysiological parameters in subjects fed with placebo or Arsenicum Album LM 0/3

($\bar{x}\pm s$)

Group	n	Blood glucose (mg/L)	Hb (g/L)	ESR (mm/h)	Total cholesterol (mg/L)	HDL-C (mg/L)
Placebo	5					
BP		998.4±31.6	88.8±6.0	8.86±3.97	1 761.5±192.4	356.1±73.4
AP		993.3±36.8	102.0±7.9	7.70±2.53	1 711.8±103.5	524.5±75.6
Arsenicum Album LM 0/3	9					
BV		896.3±74.7	67.4±3.7	16.33±6.28	1 874.7±112.1	273.6±53.5
AV		826.9±49.2	107.0±5.1▲▲	11.09±4.14▲	1 579.1±98.6▲▲	481.4±56.3▲

Group	n	LDL-C (mg/L)	Triacylglycerol (mg/L)	Creatinine (mg/L)	PCV (%)	Lymphocyte viability (%)
Placebo	5					
BP		1 204.2±136.4	1 006.1±82.0	11.7±2.5	35.80±2.05	76.53±0.69
AP		99.9±13.5△	908.5±147.8	7.3±3.8	37.40±2.95	77.06±0.54
Arsenicum Album LM 0/3	9					
BV		1 291.6±143.7	1 647.4±179.8	11.3±3.6	28.40±1.83	71.45±0.02
AV		87.9±11.6▲▲	1 271.0±168.5▲	7.5±3.3	32.70±2.26▲▲	81.25±0.98▲▲□□

In two-sample t test analysis, when $P<0.05$, null hypothesis (H_0) was accepted for most parameters i.e. differences were non-significant between AP and AV; except for lymphocyte viability, □□ $P<0.01$, *vs* AP. In paired t test, △ $P<0.05$, *vs* BP; ▲ $P<0.05$, ▲▲ $P<0.01$, *vs* BV. BV: before verum; BP: before placebo; AV: after 2 months of verum; AP: after 2 months of placebo. Hb: hemoglobin; ESR: erythrocyte sedimentation rate; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; PCV: packed cell volume.

2.4 ANA titer There was marginal improvement in ANA titer of the verum-fed subjects, as compared with that of the placebo-fed ones after 2 months (Table 6). But the difference was not statistically significant.

Table 6 ANA titer in human subjects fed with placebo or Arsenicum Album LM 0/3 against normal control

Group	n	ANA titer		
		+	−	B
Control	18	0	18	0
BP	5	4	1	0
AP	5	2	2	1
BV	9	8	1	0
AV	9	5	1	3

BV: before verum; BP: before placebo; AV: after 2 months of verum; AP: after 2 months of placebo; ANA: antinuclear antibody. +: ANA titer positive; −: ANA titer negative; B: ANA titer in borderline.

2.5 Matrix metalloproteinases In the verum-fed subjects, the band intensities were slightly lower than those in the placebo-fed subjects within 2 months of treatment (Figures 2 and 3). The expressions of both the 97 kD and 112 kD bands were of lower intensity at 2 months after drug administration (AV group) than their counterparts before drug administration (BV group).

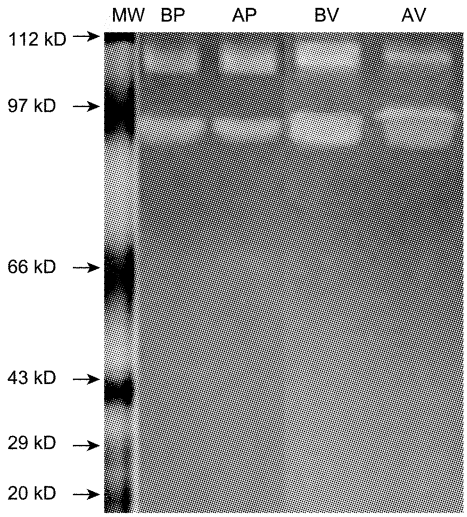

Figure 2 Gelatin zymogram showing matrix metalloproteinase expressions of blood serum samples of human subjects fed with placebo or verum

MW: molecular weight marker; BP: before placebo; AP: after 2 months of placebo; BV: before verum; AV: after 2 months of verum.

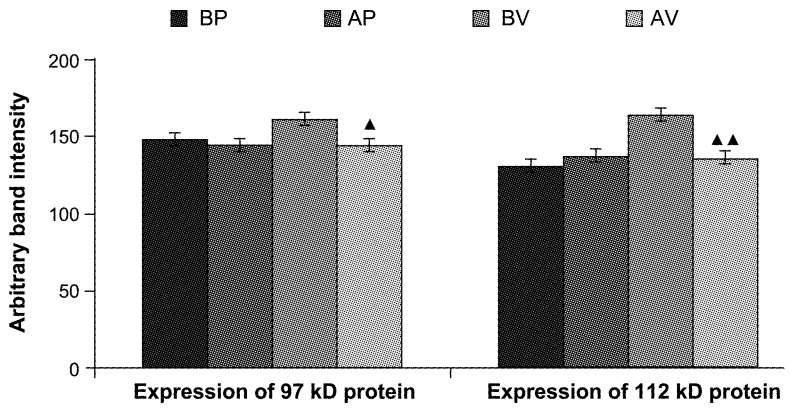

Figure 3 Quantative analysis of mean expressions of matrix metalloproteinases

▲$P<0.05$, ▲▲$P<0.01$, *vs* BV. BP: before placebo; AP: after 2 months of placebo; BV: before verum; AV: after 2 months of verum.

3 Discussion

The rationale of the parameters used has been described in detail in earlier publications on this subject by the authors[3-5, 14, 15]. In brief, activities of ALT, AST, AcP and AlkP can reflect levels of hepatotoxicity and cytotoxicity in other systems, leading to various physiological complications that may lead to typical symptoms of arsenic-induced skin diseases, anemia, cancer, or cardiovascular diseases. The victims often die of one or more of these diseases. Depletion of GSH contents and an increase in LPO provide additional support of hepatotoxicity and tissue damage. In fact, arsenic intoxication is known to disrupt the functioning of some two hundred enzymes[16]. Similarly, creatinine is a good indicator of kidney function. An increase in creatinine level indicates malfunctioning of kidney. The pathophysiological parameters used are also helpful indicators of the general state of health. Thus, a positive modulation of these biomarkers is a welcome feature that would positively indicate the ability of this remedy to render protective effects to the arsenic victims, which may have application at least to provide a temporary reprieve. In many high-risk arsenic areas, such patients with arsenicosis can be seen in increasing numbers, particularly in developing South Asia countries, who are deprived of arsenic-free drinking water, and even the minimum of medical facilities.

From the results of this pilot study, it becomes obvious that the verum-fed group showed positive ameliorating effects, like that of the other potencies of centesimal dilutions, Ars Alb 30C and Ars Alb 200C reported earlier[3-6]. Since Ars Alb LM 0/3 potency can be used for a long time and since this remedy also has protective abilities in subjects living in a village where arsenic-free drinking water facility was unavailable, this could be used as an interim measure, particularly in remote villages without having any other kind of medical facility. As a higher potency may actually be required for sustaining the ameliorative effects, it is recommended that the remedy be used under supervision of a practicing homeopath, who may suggest when the next higher potency ought to be used. Furthermore, change in symptoms may call for some intermittent remedy (may be a constitutional remedy) as based on severity of condition and totality of symptoms.

How the tiny globules soaked in the ultrahighly diluted remedy could bring about changes in so many parameters of study while the same could not be achieved by the Ars Alb LM 0/3 unsoaked globules (placebo) remain largely unknown. However, one working hypothesis advocated by Khuda-Bukhsh *et al*[17-19] is that potentized homeopathic remedies may act as a "molecular switch" to trigger specific genes that may in turn activate/deactivate a cascade of other relevant genes may be one way of explaining such inexplicable phenomenon at the state of our present understanding.

The effect of ultrahighly diluted remedies used in homeopathy has been debated over a long period of time. Although negative results have recently been reported[20] in a short time course (only 24 h) human trial, in many others positive results have also been obtained in animal models as well as in humans[3-6, 10, 14, 21-23] by using various parameters. Therefore, it is always desirable that positive results of homeopathy trials should also find a place in mainstream journals, so that unbiased and serious researchers have the impetus to carry out more independent trials to find out the truth and either confirm or refute results obtained in earlier studies.

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5 Competing interests

The authors declare that they have no competing interests.

REFERENCES

1 Chowdhury UK, Rahman MM, Mondal BK, Paul K, Lodh D, Biswas BK, Basu GK, Chanda CR, Saha KC,

Mukherjee SC, Roy S, Das R, Kaies I, Barua AK, Palit SK, Quamruzzaman Q, Chakraborti D. Groundwater arsenic contamination and human suffering in West Bengal, India and Bangladesh. Environ Sci. 2001; 8 (5): 393-415.

2 Guha Mazumder DN, Das Gupta J, Santra A, Pal A, Ghose A, Sarkar S, Chattopadhaya N, Chakraborti D. Non-cancer effects of chronic arsenicosis with special reference to liver damage. In: Abernathy CO, Calderon RL, Chappel WR. Arsenic: exposure and health effects. London: Chapman & Hall. 1997: 112-123.

3 Khuda-Bukhsh AR, Pathak S, Guha B, Karmakar SR, Das JK, Banerjee P, Biswas SJ, Mukherjee P, Bhattacharjee N, Choudhury SC, Banerjee A, Bhadra S, Mallick P, Chakrabarti J, Mandal B. Can homeopathic arsenic remedy combat arsenic poisoning in humans exposed to groundwater arsenic contamination: a preliminary report on first human trial. Evid Based Complement Alternat Med. 2005; 2(4): 537-548.

4 Belon P, Banerjee P, Choudhury SC, Banerjee A, Biswas SJ, Karmakar SR, Pathak S, Guha B, Chatterjee S, Bhattacharjee N, Das JK, Khuda-Bukhsh AR. Can administration of potentized homeopathic remedy, Arsenicum Album, alter antinuclear antibody (ANA) titer in people living in high-risk arsenic contaminated areas? Ⅰ. A correlation with certain hematological parameters. Evid Based Complement Alternat Med. 2006; 3(1): 99-107.

5 Belon P, Banerjee A, Karmakar SR, Biswas SJ, Choudhury SC, Banerjee P, Das JK, Pathak S, Guha B, Paul S, Bhattacharjee N, Khuda-Bukhsh AR. Homeopathic remedy for arsenic toxicity? Evidence-based findings from a randomized placebo-controlled double blind human trial. Sci Total Environ. 2007; 384 (1-3): 141-150.

6 Khuda-Bukhsh AR, Roy-Karmakar S, Banerjee A, Banerjee P, Pathak S, Biswas SJ, Haque S, Das D, Boujedaini N, Belon P. A follow-up study on the efficacy of the homeopathic remedy Arsenicum Album in volunteers living in high risk arsenic contaminated areas. Evid Based Complement Alternat Med. 2009 Aug 24. [Epub ahead of print]

7 Banerjee NK, Sinha N. Sinha's text book of pharmacy — a treatise on homoeopathic pharmacy. Calcutta: New Allen Publishers. 1984: 44-50.

8 Pathak S, Kumar Das J, Jyoti Biswas S, Khuda-Bukhsh AR. Protective potentials of a potentized homeopathic drug, Lycopodium-30, in ameliorating azo dye induced hepatocarcinogenesis in mice. Mol Cell Biochem. 2006; 285(1-2): 121-131.

9 Pathak S, Khuda-Bukhsh AR. Assessment of hepatocellular damage and hematological alterations in mice chronically fed p-dimethyl aminoazobenzene and phenobarbital. Exp Mol Pathol. 2007; 83(1): 104-111.

10 Pathak S, Bhattacharjee N, Das JK, Choudhury SC, Karmakar SR, Banerjee P, Paul S, Banerjee A, Khuda-Bukhsh AR. Supportive evidences for anticancerous potential of alternative medicine against hepatocarcinogenesis in mice. Forsch Komplementarmed. 2007; 14 (3): 148-156.

11 Kusum, Raina R, Verma PK, Pankaj NK, Kant V, Kumar J, Srivastava AK. Haematological profile of subacute oral toxicity of molybdenum and ameliorative efficacy of copper salt in goats. Toxicol Int. 2010; 17 (2): 82-85.

12 Bowers LD. Kinetic serum creatinine assays Ⅰ. The role of various factors in determining specificity. Clin Chem. 1980; 26(5): 551-554.

13 Kwok AK, Yeung CK, Lai TY, Chan KP, Pang CP. Effects of trypan blue on cell viability and gene expression in human retinal pigment epithelial cells. Br J Ophthalmol. 2004; 88(12): 1590-1594.

14 Banerjee A, Chakrabarty SB, Karmakar SR, Chakrabarty A, Biswas SJ, Haque S, Das D, Paul S, Mandal B, Naoual B, Belon P, Khuda-Bukhsh AR. Can homeopathy bring additional benefits to thalassemic patients on hydroxyurea therapy? Encouraging results of a preliminary study. Evid Based Complement Alternat Med. 2010; 7 (1): 129-136.

15 Banerjee P, Bhattacharyya SS, Pathak S, Naoual B, Belon P, Khuda-Bukhsh AR. Comparative efficacy of two microdoses of a potentized homeopathic drug, arsenicum album, to ameliorate toxicity induced by repeated sublethal injections of arsenic trioxide in mice. Pathobiology. 2008; 75(3): 156-170.

16 Ratnaike RN. Acute and chronic arsenic toxicity. Postgrad Med J. 2003; 79(933): 391-396.

17 Khuda-Bukhsh AR. Potentized homoeopathic drugs act through regulation of gene-expression: a hypothesis to explain their mechanism and pathways of action *in vitro*. Complement Ther Med. 1997; 5(1): 43-46.

18 Khuda-Bukhsh AR. Towards understanding molecular mechanisms of action of homeopathic drugs: an overview. Mol Cell Biochem. 2003; 253(1-2): 339-345.

19 Khuda-Bukhsh AR. Laboratory research in homeopathy: pro. Integr Cancer Ther. 2006; 5(4): 320-332.

20 Paris A, Gonnet N, Chaussard C, Belon P, Rocourt F, Saragaglia D, Cracowski JL. Effect of homeopathy on analgesic intake following knee ligament reconstruction: a phase Ⅲ monocentre randomized placebo controlled study. Br J Clin Pharmacol. 2008; 65(2): 180-187.

21 Eizayaga FX, Aguejouf O, Desplat V, Belon P,

Doutremepuich C. Modifications produced by selective inhibitors of cyclooxygenase and ultra low dose aspirin on platelet activity in portal hypertension. World J Gastroenterol. 2007; 13(38): 5065-5070.

22 Eizayaga FX, Aguejouf O, Belon P, Doutremepuich C. Platelet aggregation in portal hypertension and its modification by ultra-low doses of aspirin. Pathophysiol Haemost Thromb. 2005; 34(1): 29-34.

23 Sainte-Laudy J, Belon P. Use of four different flow cytometric protocols for the analysis of human basophil activation. Application to the study of the biological activity of high dilutions of histamine. Inflamm Res. 2006; 55(Supply 1): S23-S24.

千分之一效能白砷剂改善慢性砷中毒症状的初步临床观察

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背景:全世界有数以百万计的人饮用砷污染的地下水,而目前尚未有缓解慢性砷中毒症状的有效制剂。文献报道白砷剂 LM 0/3 作为一种顺势疗法强化制剂,对于砷中毒症状有一定的治疗作用。

目的:本研究旨在验证白砷剂 LM 0/3 对由于生活环境所致而长期饮用砷污染水的人群的慢性砷中毒症状的缓解作用。

设计、场所、对象和干预措施:本研究为双盲安慰剂对照研究,参加本研究的 28 名志愿者来自印度西孟加拉邦纳迪亚区一村落,该村无不含砷的饮用水可用,水井中的含砷量为 55～95 μg/mL。受试者分别服用药物或安慰剂,在参加试验前及 2 个月后试验结束时提供血、尿样本。另有 18 名来自无砷污染饮用水村的志愿者提供血、尿样本作为阴性对照。

主要结局指标:测量受试者血、尿样本中的砷含量,各类中毒生物标记物及病理生理指标。

结果:28 名受试者中,14 名愿意提供血、尿样本,其余 14 名退出试验。受试者的血、尿样本检测出砷的浓度升高,碱性磷酸酶及酸性磷酸酶水平升高,脂质过氧化反应及谷胱甘肽活性升高,血糖、三酰甘油、总胆固醇及低密度脂蛋白胆固醇水平升高,天门冬氨酸氨基转移酶及丙氨酸氨基转移酶水平、γ 谷氨酰转移酶及葡萄糖 6 磷酸脱氢酶含量降低,高密度脂蛋白胆固醇水平及红细胞压积降低。2 个月后,用药组的上述指标均有所改善,基质金属蛋白酶活性较安慰剂组有所降低。

结论:在尚未有更好的医疗条件和治疗手段的情况下,使用白砷剂 LM 0/3 在饮用水受砷污染地区作为暂时的治疗手段以缓解生活在这些地区的人们的慢性砷中毒症状有一定的可行性。

关键词:砷剂;顺势疗法;砷中毒;生物学标记;基质金属蛋白酶类;临床试验