Original Article


Philippe Belon¹, Pathikrit Banerjee², Sandipan Chaki Choudhury², Antara Banerjee², Surjyo Jyoti Biswas², Susanta Roy Karmakar², Surajit Pathak², Bibhas Guha², Sagar Chatterjee², Nandini Bhattacharjee², Jayanta Kumar Das² and Anisur Rahman Khuda-Bukhsh²

¹Boiron Lab, 20 rue de la Libération, Sainte-Foy-Les-Lyon, France and ²Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India

To examine whether elevated antinuclear antibody (ANA) titers reported in random human population of arsenic contaminated villages can be reverted to the normal range by administration of a potentized homeopathic drug, *Arsenicum album*, randomly selected volunteers in two arsenic contaminated villages and one arsenic-free village in West Bengal (India) were periodically tested for their ANA titer as well as various blood parameters in two types of experiments: ‘placebo-controlled double blind’ experiment for shorter duration and ‘uncontrolled verum fed experiment’ for longer duration. Positive modulation of ANA titer was observed along with changes in certain relevant hematological parameters, namely total count of red blood cells and white blood cells, packed cell volume, hemoglobin content, erythrocyte sedimentation rate and blood sugar level, mostly within 2 months of drug administration. Thus, *Arsenicum album* appears to have great potential for ameliorating arsenic induced elevated ANA titer and other hematological toxicities.

Keywords: antinuclear antibody (ANA) – *Arsenicum album* – arsenic toxicity – blood cells – blood sugar – ESR – human – homeopathic remedy

Introduction

Contamination of drinking groundwater with various toxic metal compounds including arsenic has become a menacing problem in many countries. It has affected millions of people globally distributed over some 20 countries, including over 100 million people in West Bengal (India) and Bangladesh alone (1). Prolonged exposure to Arsenic (‘As’) leads to various ailments and dysfunctions of several vital organs such as liver, kidney and lung apart from its toxic effects manifested on skin and other epidermal tissue (2). Most of the affected people in general complain of muscle and joint pains and are highly depressed with various gastric problems and general weakness. Many people look pale and anemic and seem resigned to fate. Various disease symptoms and diseases involving different organs have been reported in people of arsenic affected areas (3–5). However, no systematic survey on incidence of any systemic autoimmune disorder occurring in random populations of arsenic contaminated villages seems to have been carried out so far although some sporadic case studies have been reported (6–7). However, in one of our recent surveys, we observed an alarming prevalence of ANA-positive subjects in two arsenic contaminated villages of West Bengal (8). Moreover, efforts to reverse or alter ANA titer through administration of any orthodox medicine often bringing adverse side-effects, even though the treatment with
Methods

Selection of Work Site

The village Ghetugachhi (under Chakhda Block, District Nadia, West Bengal) is one of the worst affected and earliest known (since 1995) arsenic contaminated villages in West Bengal. Dakshin Panchpota situated ~8 km away under the same block and district is another village where arsenic has been detected in tube well waters only since 2002.

Arsenic-free water plants have been installed only since November 2002 and December 2004, respectively, in these two villages. The villagers mainly use water from the plant for drinking, but generally use other water sources for cooking and other purposes. Thus, the people of Ghetugachhi were known to be subjected to arsenic exposure for a greater length of time since detection than those of the Dakshin Panchpota village. The general symptoms of extreme fatigue, rheumatic joint and muscle pains, and gastric and urinary problems were quite prevalent in villagers of these two arsenic contaminated villages. A few people also had scleroderma and various other skin diseases. Mainly because of its proximity to the laboratory (within 30–40 km), and the availability of the arsenic-free water to the villagers, these two villages were selected as the site of our work.

Another village, Padumbasan, under Tamluk Sub-Division of Purba Medinipur district, ~160 km away from these two villages, reported to be free of groundwater arsenic (also confirmed by us by testing water samples collected from several tube wells in that village), was selected for scoring blood data which could serve as negative controls. Most of the volunteers had generally good health, albeit with minor gastric problem, but two of them complained of occasional muscle and joint pains, including one who was diagnosed with arthritis.

The Subjects

Our objectives and mission of testing the efficacy of a potentized homeopathic remedy that worked well in mice in our earlier studies were explained to the villagers at an awareness program on different aspects of large-scale arsenic contamination in several villages. Active help and cooperation were sought from these villagers to undertake this human trial. Initially the villagers did not enthusiastically volunteer. Most of them appeared to be reluctant presumably because (i) many of them simply did not believe in homeopathy; (ii) many had taboo against giving blood which, they believed, would cause them further harm; and (iii) some researchers had earlier taken blood, urine, nail and hair samples from them promising them to give effective remedies, but never came back again with either the results or the promised remedy! after great perseverance, however, some villagers agreed to undertake a blind placebo-controlled study only for 1 month, but most others maintained that they would agree to sign the ‘informed consent’ form and give us their blood samples only if they were assured that they would be given the actual drug for a long time and not any ‘placebo’. Many of the latter group of people were indeed very sick, some with visible symptoms of arsenicosis, some with serious gastric and other problems including liver and lung ailments, and a few others with bad skin lesions. Further, since there are no hospitals or clinics to care for any emergency situation, the study had to be performed under obvious limitations and was designed accordingly in the following manner.

Controlled Experiments

A group of 26 volunteers (of the same socioeconomic group as that of the village Dakshin Panchpota in district Nadia) were chosen on a random basis (as far as practicable from the willing villagers, that is!) from the village Padumbasan in Purba Medinipur district to provide data which could serve as negative control (Group I).

A group of 43 volunteers were selected from Dakshin Panchpota (Group II), who agreed to undertake double blind placebo trial (that is, each of them was allowed to choose a vial from a medicine tray containing equal number of numerically marked vials of either ‘placebo’ or ‘verum’, prepared by the associated homeopathy doctor, who was not a member of the team entrusted to observation. The content of the vials
was only deciphered at the end of the experiment by the doctor who prepared them, and was not known before either by any of the observers entrusted to take care of the different parameters of the study or to the subjects who selected their own vial; however, it transpired later that the ‘placebo’ and ‘verum’ vials were not randomly placed in the tray, which possibly should have been done, but kept merely in different rows by the associated homeopathy doctor. This limited the actual randomization process, as some volunteers simply picked up consecutive vials one after the other from the same row) only for 1 month. All the parameters of study as listed below were studied in these two groups.

The male volunteers as well as unmarried women volunteers were permanent residents of the three villages since birth, but the womenfolk married to the villagers became residents only after their marriage.

Uncontrolled ‘Verum’ Fed Experiments

Since volunteers did not agree to subject themselves to ‘placebo’ trial, but were only eager to take the ‘verum’ on their own, the next group (Group III) of 83 volunteers from Dakshin Panchpota village received only Arsenicum album-30 and their blood was drawn after 2 months of drug administration for studying the various blood parameters except for erythrocyte sedimentation rate (ESR) and PCV.

As the inhabitants of Ghetugachhi had been exposed to groundwater arsenic for a longer period than those of Dakshin Panchpota, 47 volunteers (Group IV) from this village were administered Arsenicum album-200, a higher potency, which is claimed in homeopathic doctrine to have better efficacy in case of chronic diseases (or long-standing disorders). This was performed also for the purpose of examining relative efficacy after 2 months of administration of Arsenicum album-200 and Arsenicum album-30 in two groups of arsenic intoxicated subjects. All parameters were studied in them except for PCV and ESR.

Administration of ‘Verum’ and ‘Placebo’

The potentized homeopathic drugs, Arsenicum album-30 and Arsenicum album-200 (verum) and potentized ethyl alcohol (‘placebo’, i.e. succussed ethyl alcohol-30 and ethyl alcohol-200) used in this study were prepared by Boiron Laboratory, Lyon, France following the homeopathic procedure of dilution and succussion. Sugar globules (No.20) were soaked with either verum or placebo and were given to subjects, 8 globules forming a dose, twice daily for 10 days for Arsenicum album-30 and then withdrawn for 10 days, and then given for 10 days again, and so on. However, Arsenicum album-200 was given as a single dose everyday for 5 consecutive days and withdrawn for 20 days to repeat again for 5 days, and this cycle was continued till the end of 2 months.

Methods

Blood Collection

Volunteers of different age-groups and sex were told not to take any food before drawing blood in the morning. Blood was drawn from their superficial vein around the forearm region by the routine procedure using sterile disposable syringe and needle. Blood was collected in two vials: one containing EDTA (anti-coagulant) and the other without EDTA and brought to the laboratory in flasks containing ice. In the laboratory, blood was centrifuged at 8000 g for 10 min and serum was obtained from blood without EDTA, which was used for ANA test. Blood with EDTA was also used for determination of ESR, PCV, Hb, and TC of RBC and WBC. Plasma was isolated from blood with EDTA by centrifugation at 8000 g for 10 min for the determination of blood sugar level.

ANA and Scl-70 Tests

A small part of blood serum was taken for ANA test by using an ANA Detect kit (ANA ORG 600; ORGENTEC Diagnostika GmbH, Germany) with the aid of an ELISA Reader (ELDEX 3.8, USA). This assay collectively detects, in one well, ANAs against some 20 antigens, including double-stranded DNA (ds-DNA, nDNA), histones, SS-A/Ro, SS-B/La, Sm, SmRNP, Scl-70, PM-Scl-100, Jo-1, and centromeric antigens. As ELISA test gives a better and dependable result for detection of ANA titer, this method was preferred. Blood sera of the ANA-positive subjects were also subjected to the Scl-70 antibody test by the specific anti-Scl-70 antibody kit also obtained from Germany (ANA ORG 600; ORGENTEC Diagnostika GmbH).

Other Blood Parameters

Blood with EDTA was used in a hemocytometer for TC of RBC and WBC by the routine method (improved Neubauer Hemocytometer method).

PCV was determined by the standard Wintrobe method. For determination of ESR, the standard Westergren method was followed.

For blood sugar determination, standard glucose test kit (enzymatic, GOD-POD method) obtained from Span Diagnostics Limited, India, was used and the level of blood sugar was determined by a Spectrophotometer (Pharmaspec, UV 1700, Shimadzu, Japan) at 550 nm.

Hemoglobin content was determined by Sahli’s method with the help of a hemometer (Marienfield, Germany).

Determination of As Content in Water

Arsenic content of water was determined by the standard procedure of Atomic Absorption Spectroscopy using Perkin-Elmer Analyst AA200, USA (8).
Statistical Analysis

Levels of significance between data of two fixation intervals in either placebo fed or drug fed series were analyzed by performing student’s ‘t’-test. The differences between data of subjects before feeding of drug were compared with that of data after feeding of drug for different time intervals. Further, the data of subjects living in arsenic contaminated villages were also compared with that of arsenic-free village (negative control) before administration of drug, in order to show whether the differences were statistically significant or not at the time of starting the experiments.

Results

As Content in Water Collected from Different Tube Wells

The ‘As’ content of water from three tube wells of Padumbasan village tested was found to be below the detectable limit. However, although the ‘As’ content of water collected from the arsenic-free plants of both Ghetugachhi and Dakshin Panchpota tested ‘As’ content below 10 ppb (within the permissible range of up to 50 ppb for third world countries) on an average of three tests performed on three different days, some other tube wells in both the villages had water samples measuring an ‘As’ content between 65.9 and 330.37 ppb in Ghetugachhi (10 tube wells tested) and between 94.35 and 339.38 ppb in Dakshin Panchpota village (5 tube wells tested), which are well above the recommended safe level.

ANA Titer

Out of 26 controls (negative) of Group I living in Padumbasan village (arsenic-free), only one woman having symptoms of arthritis tested ANA positive while all others tested negative (see Table 1).

The Group II controlled study for 1 month (Table 2) did not reveal any clear-cut results. There was some indication of positive alteration of titer toward negativity in the drug fed subgroup. Interestingly, in the placebo fed ones, there was also a slight decrease in the level although the change was not so remarkable.

However, in the uncontrolled Group III receiving Arsenicum album-30 for 2 months, all ANA-positive subjects tested negative. In this group, out of a total of 83 subjects (50 males and 33 females), 10 males and 6 females were ANA positive, 6 males and 2 females were on the borderline of positivity, while the rest tested negative, before administration of the remedy.

Similarly, in the other uncontrolled Group IV subjects living at Ghetugachhi village, all of the ANA-positive cases tested negative for ANA titer after 2 months of administration of the remedy, Arsenicum album-200. In this group, out of 47 volunteers (33 male and 14 female), as many as 27 males and 13 females tested positive while ANA titer of 3 males and 1 female were in the borderline of positivity before administration of Arsenicum album-200.

Scl-70 Titer

Out of a total of 56 ANA-positive cases examined, only one subject was found to be Scl-70 positive.

Table 1. Classified data on expression of ANA in relation to age and sex

<table>
<thead>
<tr>
<th>Age group</th>
<th>Padumbasan</th>
<th>Ghetugachhi</th>
<th>Dashin Panchpota</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male +ve B</td>
<td>Female -ve</td>
<td>Male +ve B</td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>0 0 0 0</td>
<td>9 1 1</td>
<td>3 1 0</td>
</tr>
<tr>
<td>20–40 years</td>
<td>0 0 18 1</td>
<td>13 1 2</td>
<td>7 0 0</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>0 0 5 0</td>
<td>5 1 0</td>
<td>3 0 0</td>
</tr>
</tbody>
</table>

Total populations studied (156): Padumbasan, 26 (Male 23, Female 3); Ghetugachhi, 47 (Male 33, Female 14); and Dakshin Panchpota, 83 (Male 50, Female 33). B = borderline.

Table 2. Reversal of expression of ANA in Group II populations from the village Dakshin Panchpota

<table>
<thead>
<tr>
<th>Age group</th>
<th>Placebo</th>
<th>Drug (Ars alb-30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP</td>
<td>AP 1M</td>
</tr>
<tr>
<td></td>
<td>Male +ve B</td>
<td>Female -ve</td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>1 0 12 0</td>
<td>0 0 1</td>
</tr>
<tr>
<td>20–40 years</td>
<td>2 0 1 0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>0 0 0 0</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

Total populations studied, 43: Placebo fed, 17 (Male 16, Female 1); Drug (Ars alb-30) fed, 26 (Male 9, Female 17); NA = blood not available (dropped out), BP = before administration of placebo; AP = after administration of placebo; BD = before administration of the drug; AD = after administration of the drug, 1M = one month.
Blood Parameters

**TC of RBC**

The mean total counts of RBC and WBC in males and females have been provided in the histograms (Fig. 1a–f).

A critical analysis of data in the controlled study (Group II) reveals that there was an elevation of TC of RBC (Fig. 1a) a little more than that of the placebo fed series after 1 month. For comparison, the data of the negative control (Group I) have also been presented.

In the uncontrolled verum fed Group III, the elevation was much more pronounced after 2 months of administration of Arsenicum album-30 in TC of RBC (Fig. 1b).

The same trend as noticed in Arsenicum album-30 fed series could also be followed in the other uncontrolled verum Ars alb-200 fed Group IV, where there was also a very distinct elevation (Fig. 1c). The females showed a little better response as compared with males in this group.

**TC of WBC**

In the controlled Group II, the single female in the placebo fed subgroup tended to show WBC more than their male counterparts and the number was even elevated after 1 month (Fig. 1d). But in the drug fed subgroup, apparently the increase of WBC count was greater in females than males.

In the uncontrolled Group III verum fed subjects, there was also a clear increase in the number of WBC after 2 months of drug administration (Arsenicum album-30) (Fig. 1e).

**Figure 1.** (a) Histogram showing changes in TC of RBC in placebo fed and drug fed groups of 1 month treatment (Ars alb-30); statistical significance levels were calculated by considering differences between data of subjects of Group I and subjects of Group II, before administration of either placebo (BP) or verum (BD-30); only significant differences have been shown: *P < 0.05; **P < 0.01; ***P < 0.001. (AP = after administration of placebo; AD-30 = after administration of Ars alb-30). (b) Histogram showing changes in TC of RBC in drug fed groups of 2 month treatment (Ars alb-30); statistical significance levels were calculated by considering differences between Group I and Group III before and after administration of the drug. (c) Histogram showing changes in TC of RBC of 2 month treatment (Ars alb-200); statistical significance levels were calculated between Group I and Group IV before administration of the drug and within Group IV before and after administration of the drug (Ars alb-200). (d) Histogram showing changes in TC of WBC in placebo fed and drug fed groups (Ars alb-30) of 1 month treatment; statistical significance levels were calculated by considering differences between data of subjects of Group I and subjects of Group II, before administration of either placebo (BP) or verum (BD-30). (e) Histogram showing changes in TC of WBC in drug fed groups (Ars alb-30) of 2 month treatment; statistical significance levels were calculated by considering differences between Group I and Group III before and after administration of the drug (Ars alb-30) and within Group III before and after administration of the drug. (f) Histogram showing changes in TC of WBC in drug fed groups of 2 month treatment (Ars alb-200) against negative control; statistical significance levels calculated between Group I and Group IV before administration of the drug and within Group IV before and after administration of the drug (Ars alb-200).
However, in the other uncontrolled group (Group IV) taking Arsenicum album-200, there was a little fall in TC of WBC after 2 months (Fig. 1f).

### PCV

The PCV in both males and females was on the lower side in the controlled group (Group II); there was only a marginal rise in the placebo subgroup. However, in the verum fed subgroup, there was a fairly clear increase in data of females, but the PCV in males actually decreased slightly after 1 month (Fig. 2).

### ESR

The ESR level in verum fed subgroup in the controlled Group II series decreased appreciably in both males and females, slightly more in females, after 1 month of taking the remedy (Fig. 3). However, interestingly, there was very little decrease observed in the placebo fed subgroup.

### Blood Sugar

In the controlled Group II, fasting blood sugar level in both the placebo fed and drug fed subgroups were initially somewhat low, and in both cases there was some elevation after taking placebo or drug for 1 month (Fig. 4a).

The base level of fasting blood sugar observed in the uncontrolled Group III before administration of the remedy was quite high at 215.72, which was found to be decreased appreciably to the normal level after 2 months of drug administration (Fig. 4b).

On the other hand, in the Arsenicum album-200 fed Group IV, the fasting blood sugar level was actually slightly elevated after 2 months of drug administration (Fig. 4c).

### Hemoglobin

In the controlled Group II, the hemoglobin content was elevated in both the placebo fed and verum fed subgroups (Fig. 5a), in both males and females.
In Group III, the hemoglobin content was relatively low in the base data before drug administration, but the level was found to be slightly elevated after 2 months (Fig. 5b). However, there was also a little elevation in the level of hemoglobin in Group IV (Fig. 5c) as well, but not as much as in Group III (Fig. 5b), in both males and females.

**Discussion**

Systemic autoimmune diseases are usually due to defects in immune regulation that results in hyperactive T and B cells. This kind of disease is best exemplified by antinuclear antibodies in systemic lupus erythematosus where pathological changes are widespread, similar to the symptoms observed in subjects exposed to chronic groundwater arsenic contamination. Autoimmune disorders are also known to cause defective red cell maturation (9), leading to diseases such as pernicious anemia and hemolytic anemia. Autoimmune antibodies are also reported to cause decline in platelet counts.

The baseline data obtained for various blood parameters indicated that, presumably because of not receiving nutritious food, most of the subjects were showing some signs of low blood count, low hemoglobin content, high level of ESR etc, which possibly made them more vulnerable to the additional effects of chronic exposure to arsenic through groundwater contamination. Long exposure to this chronic poison may be the main reason behind their testing ANA positive (8). It was quite amazing that the administration of the two potencies of *Arsenicum album* successfully reverted their ANA titers to the normal range.

Further, data also revealed that there was indeed a positive modulation of some other blood parameters which may be either contributing as a cause or effect toward the conversion of ANA titer from positivity to negativity.

Autoimmunity can affect any part of the body and cause organ specific diseases such as Hashimoto’s disease, or else may cause non-organ specific diseases, which are often called systemic autoimmune diseases (9,20,21). In systemic autoimmune diseases, the immune responses are directed toward a broad range of target antigens and involve a number of organs and tissues.

Chronic autoimmune reactions are now thought most likely to play a key role in the progressive destruction of the insulin producing B cells in the islets of Langerhans (22,23). However, a high blood sugar level in many subjects of Group III living in Dakshin Panchpota village was encountered while the same in others appeared to be more or less normal. It was, however, not known whether the elevated blood sugar was due to destruction of the insulin producing cells or not.

In SLE pathological changes are seen in the skin, such as rash on face, in kidney glomeruli, joints, serous membranes and blood vessels, similar to the symptoms seen in arsenic intoxicated subjects.

Incidentally, ideal goal for treatment of autoimmune diseases is considered to be the removal of the causative factors for the autoimmune responses. The recent therapies for autoimmune diseases are more of palliative nature than being capable of providing a permanent cure (9). Therefore, the major strategy of the treatment is generally aimed at suppressing the immune system. Corticosteroids are often used for this purpose to suppress inflammatory lesions and to prolong the life of patients. However, general immunosuppression is rather undesirable as it puts the patient at greater risk for infection or development of cancer (9,22). But homeopathic therapy is devoid of this danger because of its efficacy in ultra-low doses and its non-toxic nature (24).

ANAs are reported to be present in lower titers in several disorders that include liver diseases, leprosy, multiple sclerosis, juvenile rheumatoid arthritis, etc. (25,26). It was not precisely known whether the liver disorders commonly occurring in the
arsenic contaminated villages have in some way contributed to the elevated ANA titer found in many of them.

The Ghetugachhi population had an alarmingly high frequency of occurrence of ANA in their blood sera. Out of 47 subjects, 40 (85.1%) tested positive and 4 others were on the borderline (Table 1). In Dakshin Panchpota, a total of 16 persons (19.2%) out of 83 tested positive while 7 were in the threshold. This would possibly indicate a direct relationship of exposure-time to the development of such autoimmune disorder, as subjects of both the villages are more or less from the same economic background and mostly live on undernourished diet. But the development of autoimmune disorder in such a large scale, irrespective of being a cause or consequence of arsenic toxicity, may be attributable to the development of various other fatal diseases, which may ultimately become the cause for the large-scale premature mortality encountered in these villages. People of almost all age-groups were found to be more vulnerable; females were a little more vulnerable than males (8). Incidentally, in normal random populations, the incidence of ANA-positive cases is reported to be about 5%, as also found in the random survey of Padumbasan population. Generally, ANA-positive cases are much higher in females (9 female: 1 male), particularly in women during their child-bearing years.

Interestingly, quite a few villagers in both villages did not apparently show symptoms of inflammatory rheumatic diseases, yet they tested ANA positive, indicating its possible use in early prognosis for arsenic intoxication. Therefore, similar studies by other researchers are highly warranted and encouraged, because verification and confirmation of results could bring hope to the millions of arsenic victims and possible sufferers by way of providing an easy and affordable means, at least to lessen if not get rid of their immense suffering from groundwater arsenic contamination.

How the ultra-low doses of the homeopathic remedy could bring about such hematological and immunological changes remain unclear. But one hypothesis to explain such changes could be that signals carried by potentized homeopathic drugs might be able to trigger a cascade action of relevant genes (24) back to their normal functioning.

In view of only one subject being Scl-70 positive, further studies with other more specific antibodies such as ds-DNA, anti-centromeric antibodies, ENA etc. are needed to be conducted in future to pinpoint the exact nature of the antigen(s) being targeted by chronic arsenic intoxication.

Acknowledgements

Financial assistance for the work by Boiron Laboratory, Lyon, France, is gratefully acknowledged.

References


Received September 01, 2005; accepted December 26, 2005
Submit your manuscripts at http://www.hindawi.com