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Original Experimental Research 实验论著

Potentized homeopathic drug Arsenicum Album 30C positively modulates protein biomarkers and gene expressions in *Saccharomyces cerevisae* exposed to arsenate

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Objective: This study examines if homeopathic drug Arsenicum Album 30C (Ars Alb 30C) can elicit ameliorative responses in yeast (*Saccharomyces cerevisiae*) exposed to arsenate.

Methods: The yeast *S. cerevisiae* 699 was cultured in a standard yeast extract peptone dextrose broth medium. It was exposed to the final concentration of 0.15 mmol/L arsenate for two intervals, 1 h and 2 h, respectively. The cell viability was determined along with the assessment of several toxicity biomarkers such as catalase (CAT), superoxide dismutase (SOD), total thiol (GSH) and glucose-6-phosphate dehydrogenase (G6PDH), lipid peroxidation, protein carbonylation and DNA damage. Reactive oxygen species (ROS) accumulation, expressions of relevant stress transcription activators like Yap-1 and Msn 2, and mRNA expression of yeast caspase-1 (Yca-1) were also measured.

Results: Treatment of arsenate increased lipid peroxidation, protein carbonylation, DNA damage, ROS accumulation and expressions of Yap-1, Msn 2 and Yca-1 and decreased GSH, G6PDH, CAT and SOD. Ars Alb 30C administration decreased lipid peroxidation, protein carbonylation, DNA damage, ROS formation and Msn 2 and Yca-1 expressions and increased cell viability, GSH, G6PDH, CAT and SOD significantly ($P<0.05$), except for a slight increase in Yap-1 expression.

Conclusion: Ars Alb 30C triggers ameliorative responses in *S. cerevisiae* exposed to arsenate.

Keywords: *Saccharomyces*; arsenicals; homeopathy; reactive oxygen species; reverse transcriptase polymerase chain reaction

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The use of complementary and alternative medicine (CAM) in the treatment of various health problems is gaining importance in recent years, mainly due to the unavoidable side effects, potential allergic reactions and drug resistance of orthodox medicines. Therefore, the demand exists for alternative medicines, which, besides having potential for alleviating a wide variety of symptoms, are also holistic in nature and do not produce any adverse side effects.

Among these CAM practices, homeopathy occupies a central position in many countries, particularly in some European and third-world countries. Homeopathy uses many animals, plants, minerals and synthetic substances in a ultrahighly diluted form and is potentised by conventional homeopathic methods[1]. The use of such ultrahighly diluted remedies, which may not contain even a single molecule of the original drug substance has been the centre of controversy for a long time, because the mechanism of action is yet to be accepted on strong scientific grounds. Skeptics often ridicule the effects of ultrahighly diluted homeopathic remedies to evoke only psychological placebo effects. Therefore, for validation of the scientific basis of efficacy of such ultrahighly diluted remedies, and for understanding its molecular mechanisms, novel approaches are warranted.

One of the principles of homeopathy is "similia similibus curentur" or "like cures like"[2], which means that the symptoms that are produced by repeated feeding of any particular drug in a normal healthy person (drug proving) can also be cured in a diseased person showing the same or similar set of symptoms, by the same drug. In another homeopathic doctrine (isopathy), the ill effects of a crude toxic substance (say, arsenic) can be combated by the ultrahigh dilution of the same substance (ultrahigh dilution of arsenic or potentized arsenic). Potentized Arsenicum Album 30C (Ars Alb 30C) is an ultrahigh dilution of arsenic trioxide, which is generally used when symptoms of arsenic poisoning (like vomiting, diarrhea, thirst, loss of appetite, burning, etc.) are present in the patient[3]. Arsenic is a semimetal or metalloid that causes several health hazards including cancer. It produces drastic changes in the biochemical and genetic levels, causes DNA damage and generates reactive oxygen species[4-6].

In homeopathy, the use of potentized Ars Alb is routinely made by clinicians against symptoms of arsenic toxicity. It has been experimentally proven by atomic absorption spectrophotometric studies that arsenic molecules are hardly present in the 12th and upward potencies of Ars Alb, though its efficacy as a remedy remains demonstrable[7-10]. However, in a recent study[11], the existence of some nanoparticles of the original drug substance has been demonstrated in potentized homeopathic drugs diluted above Avogadro's limit. Potentized Ars Alb 30C and 200C have been used against arsenic intoxication in mice and human subjects with satisfactory remedial outcomes[3, 7-9, 12]. Betti *et al*[13] and Lahnstein *et al*[14] observed positive effects of Ars Alb treatment in wheat seedling growth. However, till date no research has been carried out to test its efficacy on any lower eukaryote except for the lone report published very recently[15]. However, while these workers used potencies of Ars Alb in decimal dilutions (like 17×, 18×, 24×, 28×, 30×) and studied their effects on growth of *Saccharomyces cerevisiae*, in the present study we used much higher dilutions in centesimal scale (that is, 10^{60} times diluted, much above Avogadro's limit) and used biochemical and molecular parameters for evaluation. Our primary objective was to ascertain whether such an ultrahighly diluted remedy could produce any response in arsenate-exposed *S. cerevisiae*, one of the simplest and most well-known representatives of eukaryotic cells, which forms a convenient model organism for evaluating toxic effects in human cells and tissues by extrapolation.

1 Materials and methods

1.1 Strain and growth condition The yeast *S. cerevisiae* 699 (MATa ade2-1 trp1-1 leu2-3, 112 his3-11, 15 ura3 can-100) was procured from Bose Institute, Kolkata, India and maintained as a primary culture on yeast extract peptone dextrose (2% glucose, 1% yeast extract, 2% peptone, 2% agar, YEPD) agar slants. Colonies were randomly selected from this primary culture and used for inoculation into the YEPD broth in several containers. They were grown to log phase (10^6/mL) with mechanical shaking at 30 ℃ and then subdivided into the following sets. (1) Normal control: cells grown in standard medium without any treatment. (2) Arsenate-treated: cells grown in medium supplemented with 0.15 mmol/L arsenate (H_3AsO_4, Merck, Germany). This dose was selected through a range-finding trials that showed the minimum inhibitory concentration of H_3AsO_4 for *S. cerevisiae* to be 3.5 mmol/L. (3) Positive control: potentized alcohol 30C (70% ethanol succussed and diluted) was supplemented in the medium to the arsenate-exposed cells. (4) Drug-treated: the potentized Ars Alb 30C (prepared in 70% alcohol as vehicle) was added to the arsenate-exposed cells. This drug was freshly prepared and supplied by Boiron Laboratory, Lyon, France.

All the control and treated groups of cells were then grown in the same physical condition mentioned above. Aliquots of cells from each group (10^6/mL) were taken out at 1 h and 2 h time points and different sets of experiments were carried out thrice with three replicates ($n=9$) for each group and their mean values (obtained from each of 9 sets of replicates) were compared for statistical analysis.

The experimental cultures either treated with the homeopathic remedy or its placebo were coded and not known to the observers (blinded) during observation and scoring of the data. The codes were later deciphered to know which of them actually belonged to which of the groups.

1.2 Cell viability test

1.2.1 Methylene blue staining Viability was tested by methylene blue staining. Cells were counted by haemocytometer under a compound microscope. Dead cells took up the blue colour but viable cells did not[16].

$$\text{Viable cells}=\frac{\text{Number of living cells}}{\text{Number of total cells}}\times 100\%$$

1.2.2 Methyl thiazolyl tetrazolium assay Cells from each group were harvested by centrifugation (7 000×g) for 10 min at 4 ℃ and washed twice with potassium phosphate buffer (pH 7.4). MTT (3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide) (1 mg/mL) was added to the cell pellet and incubated for 2 h with frequent shaking. Supernatant was discarded after centrifugation and dimethyl sulfoxide (DMSO) was added to the cell pellet and mixed well by vortex. After centrifugation, supernatant was collected and the optical density (OD) value was measured at 595 nm. The percentage of viable cells was determined by considering 100% viability in control untreated group.

1.3 Preparation of cell extract Cells were harvested by centrifugation (7 000×g) for 10 min at 4 ℃ and washed twice with potassium phosphate buffer (pH 7.4). The cell pellets were then resuspended in lysis buffer (50 mmol/L tris-hydrochloric acid, 125 mmol/L sodium chloride, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L ethylene diamine tetraacetic acid (EDTA)) and sonicated in cold condition. Cell debris was removed by centrifugation at 15 000 × g for 10 min at 4 ℃.

1.4 Molecular and biochemical methods

1.4.1 Evaluation of lipid peroxidation, protein carbonylation, total thiol content and protein concentration Lipid peroxidation was analyzed using the method described by Aust[17]. The amount of malondialdehyde formed in the total reaction was calculated using extinction coefficient 155 mmol/(L · cm). Carbonyl protein content was measured according to the protocol described by Lushchak *et al*[18] and calculated from the absorbance maximum of reaction product 2, 4-dinitrophenylhydrazone measured at 370 nm using an extinction coefficient of 22 mmol/(L · cm). For total thiol (GSH) assay, a modified Ellman's method[19] was followed. Cell extract was added to a reaction mixture of sulfosalicylic acid, sodium phosphate buffer (pH 8.0) and deionized water. 5, 5′-dithiobis-(2-nitrobenzoic acid) (DTNB) solution was added and the absorbance was read at 412 nm. GSH content was calculated using an extinction coefficient of 13.6 mmol/(L · cm) for DTNB at 412 nm. Protein concentration was determined by the Coomassie brilliant blue G-250 dye binding method with bovine serum albumin as the standard[20].

1.4.2 Assay of catalase, superoxide dismutase and glucose 6 phosphate dehydrogenase activities Catalase (CAT) and superoxide dismutase (SOD) activities were measured using the methods of Maehly *et al*[21] and Nishikimi *et al*[22], respectively. Glucose-6-phosphate dehydrogenase (G6PDH) activity was measured by the method of Lushchak *et al*[18].

1.4.3 Comet assay Cells were suspended in 0.7% low-melting agarose containing 2 mg/mL of zymolase 20T[23] and layered over a frosted microscopic slide. The slides, immersed overnight in lysis buffer of pH 10.0, were electrophoresed in a buffer containing 300 mmol/L sodium hydroxide and 1 mmol/L disodium EDTA, pH 13.0 for 20 min (300 mA, 20 V). The slides were then washed thoroughly with a neutralizing buffer (Tris 0.4 mol/L, pH 7.5), stained with ethidium bromide (1 mg/mL) and examined under a fluorescence microscope. The extent of DNA breakage was determined by measuring the comet tail length using the software Motic Image, China.

1.4.4 4′, 6-diamidino-2-phenylindole staining Cells were routinely fixed with 70% ethanol and incubated with 4′, 6-diamidina-2-phenylindole (DAPI) for 5 min at dark conditions, and then observed under a fluorescence microscope.

1.4.5 Estimation of reactive oxygen species Cells were washed thoroughly with sterile deionized water and then incubated for 2 h with 10 μg/mL 2′, 7′-dichloro dihydrofluorescein diacetate (H_2DCF-DA) in dark conditions. The intracellular reactive oxygen species (ROS) were measured by a flow-cytometer with an excitation wave length of 480 nm.

1.4.6 Western blot Cell lysates from each set, containing equal amounts of protein, were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a nitrocellulose membrane (Taurus Scientific, USA). The membrane was blocked for 3×30 min with 5% nonfat dry milk dissolved in tris buffer saline (pH 7.4). The membranes were then incubated overnight at 4 ℃ with monoclonal antibody for specific protein. Next, the membrane was further incubated for 2 h with goat anti-rabbit IgG as a secondary antibody. The bound antibody was detected by 5-bromo-4-chloro-3-indolyl-phosphate nitroblue tetrazolium (BCIP-NBT). Band intensity was measured by the TotalLab Software (SPSS Data Editor, version 10).

1.4.7 mRNA expression study by reverse transcription-polymerase chain reaction Total RNA was isolated from yeast cells using TRIzol reagent (Bangalore Genei, India). Total RNA 1 μg was reversely

transcribed to cDNA using oligo-dT, dNTP mix, RNase inhibitor and M-MuLV reverse transcriptase (Chromous Biotech, India). The mixture was incubated at 37 ℃ for 1 h and terminated at 95 ℃ for 2 min. Polymerase chain reaction (PCR) was performed with 5 μL of reverse transcription (RT) product in total volume of 20 μL containing 1.5 units of Taq DNA polymerase (Chromous Biotech, India), 10 × PCR buffer, 0.2 mmol/L of dNTPs, and 1 μL of each forward and reverse primers for yeast caspase-1 (Yca-1) (Table 1). These primers were procured from Bioserve, India (temperatures for the forward and reverse primers were at 62 ℃ and 60 ℃, respectively). PCR was performed on an automated thermal cycler (Applied Biosystems, USA) for 35 cycles. Amplified cDNA products were separated on 1.5% agarose gel electrophoresis in tris acetate EDTA (TAE) buffer (10 mmol/L Tris, 0.1 mmol/L EDTA, 0.55 mL acetic acid) with 0.5 μg/mL ethidium bromide and visualized under ultra-violate (UV) trans-illuminator and photographed. Densitometry was performed on a negative image using TotalLab Software. Similarly RT-PCR expression of glucose-3-phosphate dehydrogenase (G3PDH) was also studied for data normalization.

1.5 Preparation and source of Ars Alb 30C and placebo 30C The Ars Alb 30C and placebo 30C (70% ethanol) were prepared by the procedure of homeopathic serial dilutions and agitations as recommended by the French Homeopathic Pharmacopea. This involved a specific type of dilutions accompanied by agitations termed as "succussions/jerks". A total of 1 mL of 1% arsenic trioxide dissolved in 70% ethyl alcohol (the initial drug substance, called the mother tincture in homeopathy) was diluted with 99 mL of 70% ethanol and the mixture was given 10 mechanical jerks as per the traditional method to produce a potency of 1.1 mL of potency 1 and again mixed with 99 mL of 70% ethanol and agitated similarly to produce potency 2 and so on. The Ars Alb 30C and placebo 30C were procured from Boiron Laboratory, Lyon, France by using 70% ethanol of the same source.

1.6 Statistical analysis Values were shown as $\bar{x} \pm s_{\bar{x}}$ taken from 9 replicates. Data were analyzed and the significance of difference among different groups was analyzed by one-way analysis of variance. Further, the SNK-q test and Dunnette t test were conducted for analysis of significance of difference between the control group and any drug-treated groups. Results were considered significant at $P<0.05$ level.

2 Results

2.1 Cell viability After being exposed to arsenate, the percentage of living yeast cells dropped to 30% compared with the normal control group up to 2 h. Placebo-treated positive control cells also showed reduced viability, whereas significant increase in viable cells up to 5% was noticed in the arsenate plus Ars Alb 30C-treated group (Table 2).

2.2 Lipid peroxidation, protein carbonylation and GSH content Malonaldehyde (MDA) formation occurring due to lipid peroxidation reaction provides indirect evidence for lipid peroxidase activity in cells. In this experiment, arsenate-intoxicated yeast cells showed higher extent of MDA formation compared with the normal untreated one. The arsenate plus Ars Alb 30C-treated cells showed significantly decreased MDA content, signifying the extent of lipid peroxidation in those cells close to the normal cells (Table 3). Similarly, carbonylated protein formation was minimal in normal untreated yeast cells, whereas sharp increase occurred after 1 and 2 h of arsenate administration (Table 3). In comparison, the arsenate plus Ars Alb 30C-treated cells revealed significantly lower levels of protein carbonylation. In both the cases, no significant difference was noticed between

Table 1 Forward and reverse primer sequences of Yca-1 and G3PDH

Primer	Sequence
Yca-1	Forward: ATGTATCCAGGTAGTGGACGTTACACCTAC
	Reverse: CTACATAATAAATTGCAGATTTACGTCAATAGG
G3PDH	Forward: CCCACTAACATCAAATGGGG
	Reverse: CCTTCCACAATGCAAAGTT

Yca-1: yeast caspase-1; G3PDH: glucose-3-phosphate dehydrogenas.

Table 2 Cell viability tested by methylene blue staining and MTT assay

($\bar{x} \pm s_{\bar{x}}$, %)

Group	n	Percentage of viable cells (methylene blue staining)		Percentage of viable cells (MTT assay)	
		1 h	2 h	1 h	2 h
Normal control	9	98.270±0.730	98.660±0.667	100.000±0	100.000±0
Arsenate-treated	9	78.417±0.217	73.063±0.130	82.140±0.199	70.310±0.317
Positive control (Arsenate+placebo)	9	78.633±0.376	71.933±0.200	81.540±0.280	70.430±0.296
Drug-treated (Arsenate+Ars Alb 30C)	9	81.567±0.467*	76.240±0.229*	85.977±0.440*	74.467±0.740*

* $P<0.05$, *vs* positive control. MTT: methyl thiazolyl tetrazolium.

the arsenate-treated cells and the arsenate plus placebo-treated cells. Exposure to arsenate for 1 and 2 h decreased the free GSH content significantly in yeast cells compared with the normal untreated cells. Similar decrease of free GSH was also found in the arsenate plus placebo-treated cells. However, free GSH content was found to be increased significantly in the cells treated with arsenate plus Ars Alb 30C (Table 3).

2.3 CAT, SOD and G6PDH activities After 1 and 2 h of incubation with arsenate, there was a prominent decrease in CAT and SOD activities in the yeast cells. Cells belonging to the arsenate plus placebo-treated positive control group also showed reduced activity for these two enzymes (Table 4). On the other hand, Ars Alb 30C-treated intoxicated cells showed a significant increase in CAT and SOD activities. In case of G6PDH, yeast cells exhibited reduced activity after arsenate treatment for both 1 and 2 h. The placebo-treated positive control cells also showed similar results. However, there was a significant increase in G6PDH activity in the cells of Ars Alb 30C plus arsenate-treated group (Table 4).

2.4 DNA damage DNA damage was studied by single cell gel electrophoresis. The nuclei of the untreated normal cells appeared intact and round in shape under the microscope. However, the yeast cells treated with arsenate for 1 and 2 h showed many cells with damaged nuclei implying fragmented DNA, presenting long comet tails of varying sizes. Similarly, the arsenate plus placebo-treated cells also had long comet tails. On the contrary, the Ars alb 30 C-administered intoxicated cells presented short comet tails, signifying less DNA damage and fragmentation in their nuclei (Figure 1 and Table 5). The results were further verified with DAPI staining. No visible fluorescence was found in the untreated control cells (Figure 2A), signifying the normal level of chromatin condensation. Intense fluorescence, which is an indication of chromosome condensation, was observed in the arsenate-treated and the arsenate plus placebo-treated yeast cells after 1 and 2 h of incubation (Figures 2B and 2C). Lesser intensity of fluorescence was noted in the arsenate plus Ars Alb 30C-treated cells (Figure 2D) which signified a lesser extent of chromosome condensation in these cells.

Table 3 Lipid peroxidation, protein carbonylation and GSH content of different groups

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

Group	n	Lipid peroxidation (nmol/mg protein)		Protein carbonylation (ng/mg protein)		GSH content (μmol/L per 10^6 cells)	
		1 h	2 h	1h	2 h	1 h	2 h
Normal control	9	0.041±0.001	0.068±0.001	903.3±3.3	968.6±10.6	2.723±0.020	2.790±0.003
Arsenate-treated	9	0.059±0	0.079±0.001	1718.7±3.6	2580.3±17.6	2.070±0.073	2.260±0.004
Positive control (Arsenate+placebo)	9	0.060±0.001	0.078±0.001	1722.7±6.3	2610.3±11.3	2.100±0.050	2.261±0.003
Drug-treated (Arsenate+Ars Alb 30C)	9	0.045±0.001*	0.073±0.001*	1299.3±0.7*	2271.0±6.1*	2.390±0.037*	2.470±0.028*

* $P<0.05$, *vs* positive control. GSH: total thiol.

Table 4 Activities of CAT, SOD and G6PDH of different groups

($\bar{x}\pm s_{\bar{x}}$, U/mg protein)

Group	n	CAT		SOD		G6PDH	
		1 h	2 h	1 h	2 h	1 h	2 h
Normal control	9	84.51±0.50	82.83±0.92	12.47±0.23	13.14±0.23	37.4±0.4	38.2±0.9
Arsenate-treated	9	70.51±0.25	65.10+0.25	9.11±0.05	10.53±0.03	20.9±0.1	18.9±0.9
Positive control (Arsenate+placebo)	9	70.67±0.16	64.90±0.17	9.2±0.20	10.57±0.01	20.7±0.3	18.8±1.0
Drug-treated (Arsenate+Ars Alb 30C)	9	77.70±0.60*	72.43±0.26*	10.22±0.22*	12.14±0.02*	28.7±1.2*	23.5±0.4*

* $P<0.05$, *vs* positive control. CAT: catalase; SOD: superoxide dismutase; G6PDH: glucose 6 phosphate dehydrogenase.

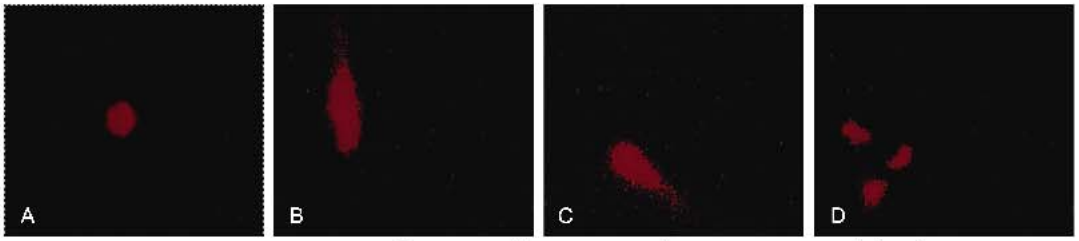

Figure 1 Comet assay of cells in different groups (Light microscopy, ×200)

Photographs of ethidium bromide-stained nuclei of control and treated cells under a fluorescence microscope after single cell gel electrophoresis. A: control; B: arsenate; C: arsenate plus placebo; D: arsenate plus Ars Alb 30C.

Table 5 Length of comet tails of cells in different groups

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

Group	n	Comet tail length (μm)	
		1 h	2 h
Normal control	9	79.40±0.30	79.30±0.38
Arsenate-treated	9	146.13±4.84	229.40±1.86
Positive control (Arsenate+placebo)	9	151.53±1.43	231.30±5.30
Drug-treated (Arsenate+Ars Alb 30C)	9	124.10±2.81*	169.03±8.96*

* $P<0.05$, *vs* positive control.

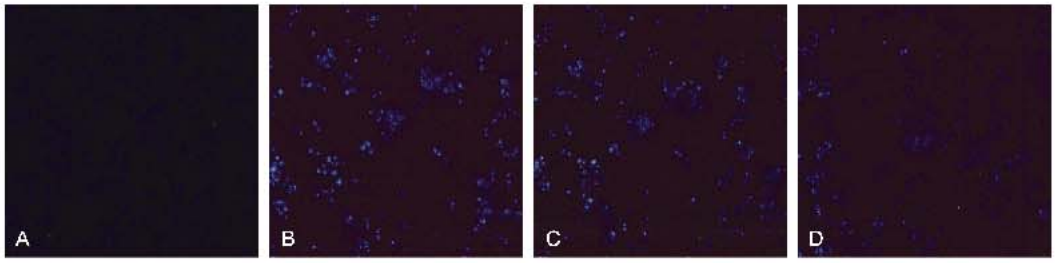

Figure 2 Chromatin condensation of cells in different groups (Light microscopy, ×400)

Photographs of DAPI-stained cells of different control and treated groups under fluorescence microscope. A: control; B: arsenate; C: arsenate plus placebo; D: arsenic plus Ars Alb 30C. DAPI: 4′,6-diamidino-2-phenylindole.

2.5 ROS accumulation ROS accumulation in cells was determined by fluorescence analysis with fluorescence-activated cell sorting (FACS). A basal level of ROS accumulation was noticed in untreated control cells. After being exposed to arsenate for 1 and 2 h, respectively, intracellular ROS accumulation was increased significantly compared with the normal cells, more so in the 2 h series. The placebo-treated control cells also showed a greater amount of intracellular ROS. In the arsenate-intoxicated cells treated with Ars Alb 30C, ROS generation was found to be at a reduced level (Figure 3).

2.6 Expressions of Yap-1 and Msn 2 proteins Western blot revealed that expression of Yap-1 protein was up-regulated after arsenate exposure for 1 and 2 h. The expression level was somewhat higher in the arsenate plus Ars Alb 30C-treated cells (Figures 4A and 4B). In case of Msn 2, significant increase was noticed after arsenate exposure, whereas the arsenate plus Ars Alb 30C-treated cells showed down-regulation of this protein to a significant extent (Figures 4C and 4D).

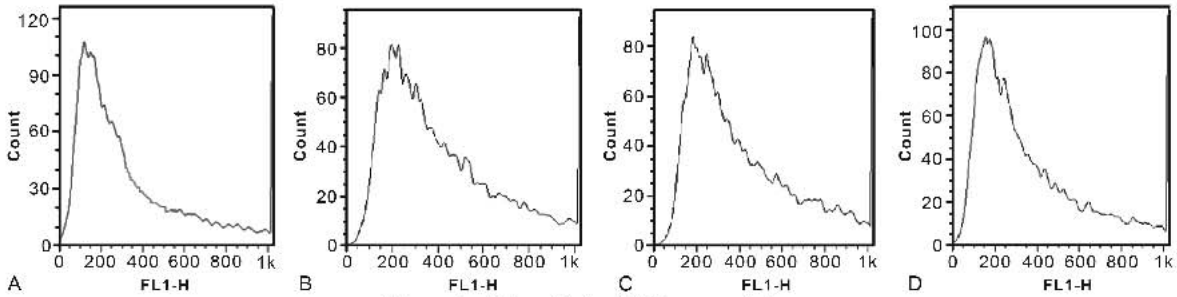

Figure 3 Intracellular ROS accumulation

FACS analysis of intracellular ROS produced by different control and treated yeast cells. A: control; B: arsenate; C: arsenate plus placebo; D: arsenate plus Ars Alb 30C. ROS: reactive oxygen species; FACS: fluorescence-activated cell sorting.

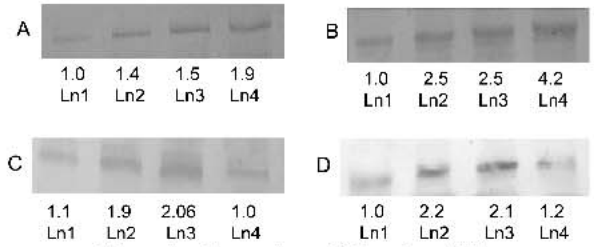

Figure 4 Expressions of Yap-1 and Msn 2 proteins tested by Western blotting

A: Yap-1 expression after 1 h treatment; B: Yap-1 expression after 2 h treatment; C: Msn 2 expression after 1 h treatment; D: Msn 2 expression after 2 h treatment. Ln1: control; Ln2: arsenate; Ln3: arsenate plus placebo; Ln4: arsenate plus Ars Alb 30C.

2.7 Expression of Yca-1 by RT-PCR Significant increase of Yca-1 expression compared with the normal untreated yeast cells was found after arsenate treatment for 1 and 2 h, but the expression decreased in the arsenate plus Ars alb 30C-administered cells to a significant level (Figures 5A and 5B), while expression of G3PDH housekeeping gene remained alike in each group (Figure 5C).

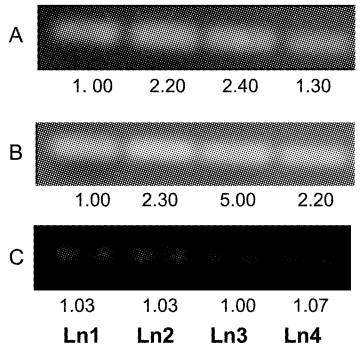

Figure 5 Expression of Yca-1 tested by reverse transcription-polymerase chain reaction

A: Yca-1 expression after 1 h treatment; B: Yca-1 expression after 2 h treatment; C: G3PDH housekeeping gene expression. Ln1: control; Ln2: arsenate; Ln3: arsenate plus placebo; Ln4: arsenate plus Ars Alb 30 C. Yca-1: yeast caspase-1; G3PDH: glucose-3-phosphate dehydrogenase.

3 Discussion

Although there is a body of evidence for how potentized doses of environmental toxins can have biological effects in animal studies[24], to the knowledge of the authors, very limited studies have been carried out to demonstrate whether a potentized homeopathic drug diluted beyond Avogadro's limit can have any modulatory effects in a primitive form of eukaryote like the yeast[25]. In the present study, the experiments were designed so as to see if the exposure of the budding yeast *S. cerevisiae* to sub-lethal doses of arsenate generated ROS and subsequent oxidative stress to the organism and also whether the ultra-highly diluted potentized remedy Ars Alb 30C could successfully combat and help the organism to tide over the stress situation to a more favorable state. The overall results obtained in this study would indicate that the homeopathic remedy clearly altered the parameters, significantly enough to demonstrate that the remedy did play a positive role in ameliorating this unfavorable condition to a much better situation. First, the viability of the organism was more than that of the placebo-treated control, and each of the relevant parameters in respect of toxicity and oxidative stress also provided supportive data. The biochemical data indicated strongly that the oxidative stress generated in the yeast was positively modulated by the homeopathic remedy, as compared with the placebo control. There were also concomitant changes in the molecular parameters which would further confirm this contention. It is known that the generation and accumulation of ROS could lead to carbonylation of protein[26]. In the present study, carbonylation was also found to be reduced along with decrease in ROS in the drug-exposed yeast cells. Similarly, an increase in CAT and SOD activities is known to be associated with the reduction of oxidative damage by scavenging superoxide radicals[27, 28]. Further, the generation and accumulation of ROS can make G6PDH a target[18]. In this study, Ars Alb 30C increased the G6PDH activity in the arsenate-intoxicated yeast cells along with a decrease in lipid peroxidation and an increase in GSH content. Similarly, DNA damage and chromatin condensation have been found to occur in lesser extent in the cells administered with Ars alb 30C. These findings were also supported by the expression pattern of the two stress proteins: Yap-1 and Msn 2. The Yap-1 transcription factor is a functional homologue of mammalian AP-1 in *S. cerevisiae*[29], and the expression of several antioxidant genes is up-regulated by Yap-1 under oxidative stress-inducing conditions. Intoxicated cells administered with Ars alb 30C showed up-regulation of Yap-1 and simultaneous down-regulation of Msn 2. In addition, Yca-1, which is known as a yeast meta-caspase[30], showed decreased expression in the Ars Alb 30C-administered intoxicated cells, which in turn denoted its inhibitory effect on the arsenate-induced programmed cell death. Besides, the overall cell viability became increased in the Ars Alb 30C-administered intoxicated cells compared with the placebo-exposed ones. Thus from the whole study it can be concluded that Ars Alb 30C, which is an ultra-highly diluted potentized homeopathic remedy, can manifest its effects on yeast cells in spite of the fact that it belongs to a primitive lower eukaryote group devoid of any centrally localized nervous system. Favorable modulation in some biochemical parameters, change of expression of proteins and genes, decreased DNA damage led the authors to believe that the protective measure exerted by homeopathic drug Ars Alb 30C in the yeast *S. cerevisiae* is due to its possible gene regulatory actions that could provide them the ability of tolerance and to reduce the arsenate stress. Further studies are warranted to understand more precisely how the initial corrective gene action is triggered and how then the cascading actions of gene expressions are regulated. However, in the meantime, extrapolation of the results of this study on yeast and earlier studies carried out on human victims living in arsenic-contaminated areas of West Bengal[3] would convincingly speak for the efficacy of Ars Alb 30C in combating arsenic toxicity in a wide variety of organisms. Therefore, this study has implications for those who are at risk of arsenic exposure through groundwater contamination or other means.

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5 Competing interests

The authors declare that they have no competing interests.

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顺势疗法药物白砷剂对暴露于砷的酵母菌的蛋白标志物及基因表达的影响

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目的:本研究旨在证实顺势疗法药物 Arsenicum Album 30C (Ars Alb 30C)是否能够改善暴露于砷酸盐的酵母菌(*Saccharomyces cerevisiae*)的各项生化指标及是否影响其 DNA 的合成。
方法:标准培养基上的酵母菌暴露于最终浓度为 0.15 mmol/L 的砷酸盐试剂中分别 1 h 和 2 h 后,检测细胞活力及其他生物活性指标如过氧化氢酶、超氧化物歧化酶、总硫醇、葡萄糖-6-磷酸脱氢酶、脂质过氧化反应、蛋白羰基化反应及 DNA 损伤情况,并对活性氧聚集情况、其他相关的应激转录激活因子如 Yap-1 和 Msn 2 的表达以及酵母细胞凋亡蛋白酶-1 进行检测。
结果:暴露于砷酸盐中的酵母菌,其脂质过氧化反应、蛋白羰基化反应、DNA 损伤、活性氧聚集及 Yap-1、Msn 2 和酵母细胞凋亡蛋白酶-1 的表达均有所升高,而过氧化氢酶、超氧化物歧化酶、总硫醇及葡萄糖-6-磷酸脱氢酶的水平均有所降低。与对照组比较,与 Ars Alb 30C 共培养的细胞以上指标均有明显改善($P<0.05$),只有 Yap-1 的表达未见明显降低。
结论:顺势疗法药物 Ars Alb 30C 能够激活暴露于砷酸盐的酵母菌的自我调节能力。
关键词:酵母菌属;砷剂;顺势疗法;活性氧;逆转录聚合酶链反应

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