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Short Communication
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Sodium Arsenite Intoxicated *Escherichia Coli* Differ in Protective Responses Induced by Two Different Ultra-Highly Diluted Substances: A Commentary on our Published Research

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Abstract

Arsenic is a toxic metalloid distributed in the whole environment causing stress and various health hazards to living organisms, necessitating them to evolve precise mechanisms for arsenic detoxification/extrusion. The bacterial model *Escherichia coli* actively extrude arsenic via ATP (Adenosine triphosphate) mediated transporter proteins primarily by converting glucose into ATP. In this commentary, we analyzed the relative protective responses elicited by Sodium arsenite intoxicated *E. coli* induced by two ultra-highly diluted and succussed substances, namely, Arsenicum Album 30C and Glucose 30C derived by homeopathic procedure of dilutions (by a factor of 10^{60}) and succussions of the same toxic substance, Arsenic trioxide, and glucose, the metabolic source of high energy (Adenosine triphosphate or ATP), respectively. This commentary highlights the outcome of how *E. coli* responded differently to these two ultra-highly diluted agents in terms of some relevant parameters of study like glucose uptake, hexokinase and glucokinase activities, ATP, arsenic concentrations and expulsion, and certain gene expressions as reported in our two earlier studies.

Keywords: Sodium arsenite; Ars and permease genes; Glucose uptake; Hexokinase; ATP; Arsenic expulsion

Short Communication

Arsenic is one of the toxic metalloids present in the environment. Its trioxide form (arsenite or As III) is more toxic than its pentoxide form (arsenate or As V). Arsenic intoxication with As III produces immediate acute toxic effects in all living organisms. In view of its ubiquitous presence in the environment, all living organisms are at risk of being exposed to arsenic intoxication that can produce physiological stress and health hazards [1] and the higher animals like mammals including human show manifestation of the toxic effects by way of severe vomiting and strong diarrhoea.

One of the main working principles of homeopathy advocates "like cures like" - meaning thereby that the toxic symptoms like severe vomiting and diarrhoea induced by some toxic substance can be cured by the ultra-highly diluted and succussed solution of the same substance when administered in ultra-high dilutions and in micro doses [2]. After conducting a large number of experiments on the efficacy of the ultra-highly diluted Arsenicum Album 30C (prepared from serial dilutions and succussions of 1% Arsenic trioxide by a factor of 10^{60} times in multiple steps) in mammalian model mice (*Mus musculus) in vivo* [3-9] against arsenic poisoning deploying various toxicity biomarkers, we became interested to examine if the single-celled model of lower organisms (prokaryotes), as for example, *Eschericia coli*, with a simple genetic system exposed to sub-lethal toxic dose of sodium arsenite could also respond favourably to Arsenicum Album 30C (Ars Alb 30) and also to ultra-highly diluted Glucose 30C (Glu 30) towards arsenic entry/expulsion.

E. coli is known to have a defensive mechanism against arsenic toxicity and if arsenic enters inside the cell overpowering the resistance, the bacteria expel it by active transport with the help of an arsenic pump utilizing the energy derived from glucose metabolism. The present commentary is primarily based on two of our published research [10,11] that dealt with the molecular mechanism through which *E. coli* get rid of the induced arsenic toxicity and discussed the role of two ultra-highly diluted agents, Arsenicum Album 30C and Glucose 30C in the process of arsenic elimination from *E. coli*.

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All standard methodologies were used maintaining proper controls in these reported works.

The results obtained were quite interesting as *E. coli* were found to respond in two different ways in response to the treatment with the two ultra-highly diluted substances. In As-treated *E. coli*, glucose uptake, intracellular ROS, lipid peroxidation and DNA damage increased along with decrease in the specific activity of hexokinase, SOD and catalase, intracellular ATP and free GSH contents, membrane potential and growth and there was an increase in expression level of ars B gene. Ars Alb 30 administration reduced arsenic toxicity in *E. coli* by inhibiting generation of reactive oxygen species (ROS) and increasing tolerance to arsenic toxicity and growth while the placebo could not induce/modulate any change.

In the other study for examining the effects of Glu 30, arsenite exposed *E. coli* also exhibited an increase in the glucose uptake along with decrease in the specific activity of hexokinase and glucokinase, intracellular ATP, membrane potential and corresponding increase in the gene expression level (RT-PCR and Real-Time PCR) of glucose permease. Glucose uptake increased further by addition of 1%, 3% or ultra-high diluted glucose in the medium, but not by the placebo. The detailed molecular mechanisms through which *E. coli* manage the problem of arsenic intoxication has been discussed in detail in these two papers [10,11], and the elaborate roles played by the ars operon comprising mostly three or occasionally five functional genes, arsRBC, or arsRDABC, have been clearly elucidated [12] that involved frequently plasmid-encoded efflux systems [13].

In *E. coli*, plasmid R773 is believed to confer resistance to arsenite by encoding an anion pump that helps in extrusion of arsenite from the cells [14]. Chemical energy is needed for efflux and this is obtained from breakdown of ATP to ADP. The pump is localized in the inner membrane of *E. coli*. It is composed of two types of subunits, the Ars B protein [15-16] and the Ars A protein, an oxyanion-stimulated ATPase [17]. The Ars pump is a novel anion-translocating ATPase that is not a member of any of the well-characterized cation or solute translocating ATPases [18]. Since this pump needs constant high energy supply (in form of high energy phosphate "Pi") by converting ATP into ADP for expulsion of arsenic from within the cell, ATP is in constant demand and the primary source of the ATP being from glucose metabolism, uptake of more glucose available in the medium will be deemed useful and therefore in great demand to compensate the 50% loss of production of the end product of glycolysis, that is , the pyruvate, that is converted to ATP (reduced from 8 mole to 4 mole per one mole of glucose) in arsenic intoxicated *E. coli*. Ars Alb 30 administration resisted arsenic from entering cell through regulatory expression of the *ars* genes, particularly by over-expression of ars B and ars C genes (for arsenic tolerance and pumping out arsenic) ,and also promoted the entry of glucose to some extent through modulating hexokinase and glucokinase activities. On the other hand. Glu 30 was found to mostly promote glucose entry through over-expression of permease genes and also of Ars A gene responsible for enhancing ATPase activity necessary for producing high energy phosphates that can be utilized for arsenic expulsion. Glucose uptake was increased by addition of 1%, 3% or ultra-high diluted Glu 30 in the medium, but not by the placebo (Alc 30); thus Glu 30, which had previous contact with glucose molecules appeared to mimic the action of the real glucose supplementation, indicating possibility of their carrying "molecular imprints" of glucose molecule, presumably in form of some of their nanoparticles or nano-associates [19,22]. The addition of placebo (Alc 30C, without any previous contact with either arsenic trioxide or glucose) to standard medium did not make any significant difference in any of the parameters studied. The results thus validated the efficacy of ultra-high dilutions commonly used in homeopathy, and though physico-chemically ultra-high dilutions of Ars Alb 30 or Glu 30 can not be easily distinguishable, the bacteria could react differently to the ultra-high dilutions in respect of the physiological/biochemical/gene-expression activities in these organisms with genetically simple systems and devoid of any nervous system, indicating thereby that intermediation through nervous system for executing a precise gene expression is not indispensible and can be possible without its involvement in the lower organisms. This can give a clue that if the molecular mechanism of action is considered to be the same in all living organisms, and not different in the lower and higher animals, the "gene regulatory hypothesis" first proposed by Khuda-Bukhsh [23] to explain the mechanism of biological action of potentized homeopathic drugs stands as the most plausible of all hypotheses proposed so far, which very recently has also been supported by other prominent workers [24,25].

Further, our initial observations of UV spectrophotometric analysis revealed that the verums differed significantly ($p<0.001$) in their absorbance values at all nm between 200 and 295 nm (determined at an interval of 5nm) from that of the alcohol placebo, arousing further interest in this challenging area of research.

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