

**10**

## **Randomized placebo-controlled trial to evaluate the effectiveness of selenium in the treatment of arsenicosis patients**

### **A. Momin**

Department of Dermatology and Venerology  
Dhaka Medical College  
Dhaka.

### **Shah Keramat Ali**

Department of Clinical Nutrition  
Institute of Nutrition and Food Science  
University of Dhaka  
Dhaka.

### **M. Mujibul Haque**

Department of Dermatology and Venerology  
Dhaka Medical College  
Dhaka.

#### **Abstract**

To evaluate the effectiveness of selenium in the treatment of arsenicosis, 100 patients were treated with 100 µg of selenium per day for 12 months. Another 100 patients received potato starch as placebo. At the end of treatment 13 patients from each group were drop-out. All the patients were encouraged to drink surface water which was free from arsenic contamination. The amount of arsenic in hair, nail and urine were estimated using hydride generator atomic absorption spectrometry to assess the arsenic load. The concentration of total arsenic in hair, nail and urine were reduced significantly after administering selenium. Long-term treatment of selenium did not show any adverse effects. This study suggests that selenium may be useful for the treatment of arsenicosis patients.

### **Introduction**

The poor dietary selenium intake in Bangladesh where arsenicosis is occurring and excessive selenium excretion owing to selenium/arsenic complex may add to the likelihood of arsenic being more toxic and carcinogenic over time (Spallholz, 2004). The cytotoxic effect of arsenic can

be prevented through short term dietary supplementation by selenium in mice *in vivo* which is of significance in protecting against the widespread toxicity observed in human populations exposed to arsenic through drinking water from contaminated deep tube wells in West Bengal and Bangladesh (Biswas et al., 1999). Recently, an animal study showed significant reduction of arsenic in the different tissues and vital organs of the rats which revealed the importance of selenium intervention in arsenicosis. It may be considered that antioxidant therapy with controlled dose of selenium is of benefit in reducing the arsenic accumulation and its toxicity. A placebo-controlled clinical trial with selenium and antioxidant in arsenicosis, where they found encouraging result in reducing the arsenic load from the tissue but they would not advocated for mass use (Bangladesh Arsenic Control Society, 2003).

It appears that arsenic obviously has inhibitory effect on the antioxidant enzymes containing selenium by reacting with sulfhydryl (-SH) group. This inhibitory impact has been observed in increased arsenic accumulation. Again, reduced glutathione is essential for metabolism and excretion of arsenic at the same time for synthesis and activity of selenoenzymes. There might be a competitive inverse relationship among arsenic, selenium and glutathione.

Selenium might be a suitable agent to reduce arsenic accumulation after chronic exposure for a number of reasons i.e. there are a number of possible points and mechanism for metabolic interaction between arsenic and selenium which include competition for the methyl donor, S-adenosylmethionine, competition for reduced glutathione (GSH) and inhibition of glutathione reductase by a number of arseno-glutathione complex (Kenyon, 1997). Styblo and Thomas (1995) reported that like arsenicals, selenite has been shown to react with GSH to form a seleno-di-glutathione complex. This complex is metabolized by glutathione reductase. It has also been reported in a study that arsenic, platinum and gold containing drugs significantly influence the fate of exogenous selenium, whereby they may adversely affect the availability of selenium which is essential element for the synthesis of selenoenzymes (Gregus et al., 2000).

Moreover, both selenium and arsenic interact extensively with sulfhydryl (-SH) groups in tissues, it is possible that arsenic elimination is delayed in selenium-deficiency because there could be more target -SH groups for arsenic to react with because selenium intake is low (Kenyon, 1997). Again the *in vitro* uptake of selenite by intact rat erythrocyte was found to be

proportional to cellular GSH concentration. Release of selenium was dependent upon a reaction catalyzed by glutathione reductase possibly the reduction of seleno-di-glutathione complex as reported by Styblo et al., (1997).

If there is excess arsenic in tissues, GSH is likely to be saturated in order to metabolize arsenic and defending cells from oxidative stress. Consequently, decreased amount of GSH would have interfered the selenium availability and as a result antioxidant selenoenzymes would be decreased. On the contrary, if sufficient selenium is available to the tissues, the -SH group would not be left free for arsenic to react with and there would be abundance of antioxidant enzymes to counteract the per-oxidative stress by arsenic.

There is no specific treatment widely recognized for chronic toxicity of arsenic. Chelating agents, vitamins, high nutritional diet like spirulina, all these have limitations in different grounds. Above all, it should be remembered that people with clinical manifestations are not only the sufferer but millions of people who do not present with features of arsenicosis are also highly vulnerable to the more serious and delayed health effects in the long run. Thousands of people may develop lung cancer, bladder cancer, liver cirrhosis etc. if the silent accumulation of arsenic is not prevented. The aim of the study should not be limited within symptomatic relief or chelation therapy or rich food, but should be reversible and competitive to the arsenic accumulation and toxicity even if exposed long time ago.

We found interest to evaluate the role of selenium in the treatment of arsenicosis patients and accordingly we have designed in a randomized placebo-controlled trial with selenium to observe the distribution and accumulation of arsenic in tissue level in patients and to see the effect of selenium to counteract this accumulation for quick relief of symptoms and as effective remedies. We believe that a dietary supplement is feasible as well as inexpensive and may help to turn the heat off of a potentially massive health crisis in an already impoverished place. In the present study we examined whether dietary selenium is effective to counteract the accumulation of arsenic in tissues (hair and nail) after chronic exposure.

### **Method and Materials**

*Study areas:* The study area was carried out at Muradnagar Upazilla under Comilla District (about 110 km southeast from Dhaka) and Chatkhil Upazilla under Noakahali District (about 170 km southwest from Dhaka). These areas are hyper-endemic arsenic zone according to

British Geological Survey report. A large number of people of these two Upazillas are drinking arsenic contaminated water for a long period of time.

*Duration of study:* This prospective randomized double-blind trial was carried out during the period from November 2004 to June 2006.

*Study population:* During the field visits, consultation camps were organized where people have been invited for motivation and free arsenic testing. After mass campaign 560 people were gathered at a public center. Village leaders, local non-government organizations and staff and students of the local college, assisted recruitment of the subjects. Arsenicosis were diagnosed clinically and by spot urine examination for arsenic. All adults, both male and female with history of exposure to arsenic contaminated drinking water for more than 6 months and signs/symptoms of arsenicosis were included. Exclusion criteria included patients not exposed to arsenic, no clinical feature of arsenicosis, patients refused to give consent, patients not having arsenic in urine, patients known to have received vitamins and minerals from local doctors for any condition, patients having concurrent illness like malaria, tuberculosis or other chronic illness, history of smoking, alcohol intake or taking hepatotoxic drugs. Pregnant and lactating mother were also excluded. About 200 patients were selected randomly from the sampling area.

*Development of questionnaire:* A questionnaire was developed to collect information on socio-economic situation, drinking water (source, duration of use), age and gender of participants, duration of skin manifestation, etc. The questionnaire included the option to measure blood pressure of respondents, take height and weight, and collected urine, blood, water, hair and nail samples of selected study population. The questionnaire was pre-tested and finalized after incorporation of feedback.

*Interviewers:* Six interviewers were selected from the local community. The interviewers received one day orientation training. The principal investigator provided hands on training to the interviewers during field-testing of questionnaire. The principal investigator supervised the field testing procedure and gave on the spot feedback to the trainees.

*Ethical issue:* The study was approved by the ethical review committee of the Bangladesh Medical Research Council (BMRC). Before entering the study, each patient was informed about the purpose of the study. Voluntary written consent was taken from each patient and then the subject was asked to appear for interview. All the information was kept strictly confidential for research purpose.

*Collection of data:* Each patient was given an identification number and randomly assigned into one of the two treatment groups (placebo or selenium) following a computer generated table of random numbers. Anthropometric measurements such as height and weight were measured for each of the participants following standard procedure and recorded in the specific questionnaire. The body weight was measured on standing position by bathroom scale on barefoot and light clothing and recorded to the nearest 0.1 kg. Height of the subjects were measured barefoot in standing position with an especially wooden scale and recorded to the nearest 0.1 cm. On completion of socio-demographic and anthropometric data hair, nail urine and blood samples were taken from each patient. During data collection each subjects was asked about his/her socio-economic condition and knowledge about arsenic related problems, along with related information.

*Collection of samples:* Urine and blood samples were collected before and at the end of 4<sup>th</sup>, 8<sup>th</sup>

and 12<sup>th</sup> months. All the samples were obtained during the first week of the month. Only hair and nail samples were collected at the beginning and at the end of study period. Samplings of their drinking water were also done at the beginning and at the end of study period. All the samples were transported to Dhaka by next 24 hours for laboratory analysis in frozen containers and stored at -20°C until analysis was carried out.

*Hair: Scalp hair from the neck region was selected to collect for estimation of arsenic. About 2 cm in length of hair had been put together by index and thumb finger of left hand standing behind the patients and then cut off by a sharp scissor by right hand from 1-2 mm above the scalp skin. The cut off hair had been stored to a plastic container with proper labeling such as identification number of the patient, date of collection, nature of the specimen.*

*Nail: The finger and toe nails of patients were collected for the estimation of arsenic. About 100 mg of nail has been collected by a sharp nail cutter to a white paper which is then transferred to a plastic container for storage with proper labeling such as identification number, date of collection and nature of specimen.*

*Urine: Freshly voided urine samples (20-30 ml) were collected from each patient at the spot in a pre-washed, polyethylene, screw-capped bottles. Immediately after collection, the pot had been tightly corked and was labeled with a mercury-signing pen properly which include identification number of the patient, date of collection and the nature of specimen and stored in a salt-ice mixture. All urine samples were transported in a salt-ice mixture during the collection of them in field and the samples were kept frozen during the return to laboratory. Urine was used for estimation of total arsenic.*

*Blood: After collection of hair, nail and urine the patient was asked to lie down in a bed to collect the blood sample. A tourniquet was tightly bound over the mid arm of right hand of the patient. With hexisol lotion the area of the flexor surface of elbow joint had been cleaned properly. Patient was asked to clench his fist to tighten the area, which gave a good engorgement of the vein of the area. Then by sitting besides the patient, about 5 ml blood was drawn with a gentle prick from the engorged brachial vein through a 5 ml disposable sterile plastic syringe and which immediately transferred to a sterile test tube. The prick area of the patient was sealed with a band aid tape and then the patient was advised to get up and collect his allocated drugs. The blood in the test tube now labeled with identification number and centrifuged by a centrifuge machine at 3,000 rpm for 3 minute. The supernatant serum was collected from the centrifuge tube in a sterile eppendroff tube which was then labeled with a mercury-signing pen properly for identification which includes identification number, date of collection and nature of the specimen. Then this eppendroff tube containing serum was kept in a frozen containers containing salt-ice mixture for transport to the laboratory for various biochemical tests. Blood was used for estimation of selenium.*

*Drinking water: A 100 ml bottle was supplied to each patient to bring drinking water during the next visit. All these samples were properly labeled with the identification number of the corresponding patients.*

A local office-cum-clinic was setup in the area to facilitate diagnostic and patients care activities.

*Treatment procedure:* After randomization, the attending patients were grouped in two groups

of equal 100 patients. Group A was given tablet selenium 100 microgram per day and group B was given placebo containing potato starch orally for 12 months. The trained field workers under supervision of principal investigator provided the specific treatment to the patients. Selenium in a solid tablet form, named 'Selenomax' (Manufactured by Nutrition 21, distributed by Arbor Drugs Inc, Troy MI, USA) packed as 100 tablets in a sealed bottle. Each tablet containing 200 µg selenium as high selenium yeast rich l-selenomethionine, without any artificial color or preservatives and supported by the clinical research. Placebo containing potato starch tablet of same size, shape and color without smell manufactured by The Acme Laboratories Ltd, Dhaka, Bangladesh. All the drugs were identical in appearance and taste. Treatment A or B was being blindly coded for selenium alone or placebo respectively. Neither the patients nor the investigators knew the binding codes, which was kept confidential. The codes were opened only after the data analysis was complete. The assigned treatment (A or B) was written in a piece of paper, which was preserved in a sealed non-opaque envelope. The envelope was opened only at the time of entry of a newly recruited patient. The drugs were delivered to each participant in a sealed air tight plastic bottle. Only code number was written on each bottle. Each bottle was packed earlier with 15 tablet of the respective group of drugs, which were kept confidential. Neither the investigator nor the patients knew the intervention groups. Each patient was instructed to swallow half of the tablet daily orally with a glass of water. The trained field workers under supervision of principal investigator, for 12 months, provided the specific treatment to the patients. Each patient was instructed to visit the camp every month in order to receive the drug. While receiving the drug, the patient had to bring the previously used containers in order to check the compliance. During the study period the patients were advised not to receive any other drug(s). Any patients were not allowed to drink arsenic contaminated water throughout the study period rather they are encouraged to take surface water.

The effect of medication was assessed in study groups through clinical and biochemical examination. All relevant data was noted.

*Estimation of total arsenic:* Total arsenic was estimated from hair, nail and corresponding urine. Estimation of arsenic was also done from their drinking water to confirm that patients were not re-exposed to arsenic during the study period.

*Hair and nail:* Hair and nail samples were washed twice with acetone and then with de-ionized water, air dried, and, then weighed. The dry hair/nail sample was weighed 100 mg and transferred into 50 ml polyethylene digestion tube, 2 ml high purity nitric acid was added and the samples were soaked in acid for at least 12 hours before the tube was heated in a preheated hot block heater (Environmental Express Co., USA) at 120°C for 1 hour. After initial digestion a few ml of 30% hydrogen peroxide was added and the sample was heated for additional half an hour to give a transparent solution. Finally, the solution was diluted quantitatively to a fixed volume 50 ml and the solution was analyzed for arsenic by continuous flow hydride generation atomic absorption spectrometer (HG-AAS).

*Urine:* All the urine samples were directly analyzed without acid digestion. Pre-treatment with l-cystine and reduction with potassium iodide and ascorbic acid were done for 45 minutes prior to analysis (Guo, 1997).

All biological tissues hair, nail, and serum, are digested in high purity acids and pretreated with reductants prior to analysis. A quartz T-tube mounted on a burner in an atomic absorption spectrophotometer (Buck Scientific, USA, Model 210 VGP) will serve as an

atomization cell for arsine vapor. The hydride generation method in this study using a reductant such as, sodium borohydride (in presence of acid and other reagents) to bring down all arsenic to  $\text{As}^{3+}$  state in the ground water. A peristaltic pump is utilized in mixing the sample, hydrochloric acid and the borohydride in a reaction vessel. The effluent then passes through a gas-liquid separator. The arsine gas is purged from the reaction vessel by a flowing argon gas and air to the atomization source for continuous mode detection. Absorbance of light is proportional to the concentration of arsenic in water. The atomic vapor in the heated cell absorb the resonance energy from the arsenic hollow cathode lamp beam (wavelength  $\lambda = 193.7$  nm) to provide the analytical signal. Prior to introduction of the vapor for thermal decomposition, the vapor passes through a moisture trap. The peristaltic pump and the set up allow on-line continuous hydride generation and fast arsenic detection in all samples.

*Estimation of selenium:* The amount of selenium in blood was estimated using atomic fluorescence spectrometry with hydride generator with selenium cathode at 196.1 nm wavelength. In brief, 250 microgram of serum was transferred into 50 ml polyethylene digestion tube, 2 ml high purity nitric acid was added and the samples were soaked in acid for at least 30 minutes, before the tube was heated in a preheated hot block heater (Environmental Express Co., USA) at 115°C for half an hour. After initial digestion 6 drops of 30% hydrogen peroxide was added and the sample was heated for additional half an hour to give a transparent solution. Then, 06 ml of high purity hydrochloric acid was added to the digested materials and the sample was again heated for half an hour more at 115°C. Finally, the solution was diluted quantitatively to a fixed volume (10 ml) and the solution was analyzed for selenium by continuous flow hydride generation Atomic fluorescence spectrophotometer (HG-AFS; PS analytical 10.055 Millennium Excalibur, England) with millennium software version 1.52.

Other laboratory tests were done which included liver function test (ALT, alkaline phosphatase), random blood sugar, and urine for routine examination and electrocardiogram.

*Quality assurance:* Quality assurance measures were maintained including calibration with NIST reference samples, use of blanks and replicates. For method validation, standard water from NIST (SRM 3103a), and standard hair from IAEA (IAEA-085, IAEA-086) were periodically analyzed for arsenic. Calibration of the machine were made daily with fresh standard solutions of analytical-grade arsenic from Chem Service (coefficient of variation < 5%). For each specimen, three replicates were taken. Non-detects were assigned a value one-half the detection limit.

The atomic absorption spectrophotometer is calibrated everyday with standards prepared from high purity  $\text{As}_2\text{O}_3$  (Aldrich Chemical, USA) or stock solutions from Perkin-Elmer Co. (USA). For quality control (QC), the QC standards and reagent blanks are analyzed at regular intervals. The QC checks are achieved by inserting the QC standards and reagent blanks during the continuous on-line analysis of samples. All standards and the reagents are prepared fresh daily. Distilled deionized water is used in all steps of reagent preparation and analysis. Standard reference materials (SRM 1643d, SRM 1640) from the National Institute of Standards and Technology (NIST, USA) are used as controls for precision and accuracy check of data and validation of analytical methodology.

## Result

In this study about 200 patients were selected but at the end 26 patients were drop-out, so the drop-out rate was 13%. Majority (129) of the study subjects were within 20-49 years and most of the subjects were female (112). The mean age of both the groups was more or less same ( $35.92 \pm 11.83$  years in selenium group;  $36.83 \pm 13.08$  years in placebo group). Mean BMI before

**Table 1:** Demographic characteristics of study population

	<i>Selenium (n = 87)</i>		<i>Placebo (n = 87)</i>	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Age of the patient (year)	35.9	11.8	36.8	13.0
Monthly income (Taka)	2782	3340	2459	2810
Family size	6.9	2.8	6.0	1.8
Duration of drinking water use (year)	21.1	11.4	21.9	12.9
Duration of cooking water use (year)	25.4	10.5	27.5	11.3
Body mass index (before)	20.3	3.5	20.5	3.2
Body mass index (after)	14.7	10.1	18.0	8.7

intervention in selenium group were  $20.3 \pm 3.5$  and placebo group  $20.5 \pm 3.2$  but after intervention it was  $14.7 \pm 10.1$  and  $18.0 \pm 8.7$  in selenium and placebo group respectively.

About 33.3 % subjects were drinking contaminated water initially and finally this rate dropped down to none. Table 2 shows that more than 94% cooked their meal with surface water, which we have found free from arsenic. But more than 87% were drinking arsenic contaminated water from shallow tube well water. The duration of using drinking water was  $21.1 \pm 11.4$  years (range 11.5-35.6 years).

**Table 2:** Percent distribution of source of water for drinking

<i>Source of water</i>	<i>Drinking</i>		<i>Cooking</i>	
	<i>Selenium (%)</i>	<i>Placebo (%)</i>	<i>Selenium (%)</i>	<i>Placebo (%)</i>
Deep tube well	6.9	10.3	3.4	1.1
Shallow tube well	86.2	87.4	2.3	2.3
Pond	4.6	2.3	94.3	96.6
Filter/boiling	2.3	00	-	-

Cardinal sign showed by the patients were melanosis, leucomelanosis and keratosis (Table 3).

**Table 3:** Distribution of skin manifestation

<i>Skin sign</i>	<i>Selenium</i>		<i>Placebo</i>	
	<i>Number</i>	<i>%</i>	<i>Number</i>	<i>%</i>
Melanosis	87	100	86	90.9
Leucomelanosis	82	94.3	85	97.7
Keratosis	77	88.5	83	95.4
Fissure on hand and foot	14	16.1	6	6.9
Gangrene and hemorrhage	2	2.3	0	
Non-healed ulcer ( Bowen's, squamous cell carcinoma, basal cell carcinoma)	8	9.2	10	11.5

On urine examination, no albumin was found in the study subjects before and after intervention and blood glucose level also found normal. Liver function test showed no abnormality after intervention.

Table 4 shows that nail, hair and urine were loaded with arsenic initially. But finally after intervention significant changes occurred in selenium group. None of the patients showed any adverse effect during the study period.

**Table 4:** Concentration of arsenic in nail, hair and urine; and selenium in serum

<i>Parameters</i>	<i>Group</i>	<i>n</i>	<i>Before</i>	<i>After</i>	<i>p value</i>
<i>Concentration of arsenic</i>					
Nail ( $\mu\text{g/g}$ )	Selenium	48	$3.4 \pm 3.0$	$2.2 \pm 1.9$	0.01 (S)
	Placebo	56	$2.7 \pm 3.1$	$3.2 \pm 3.4$	0.19 (NS)
Hair ( $\mu\text{g/g}$ )	Selenium	52	$1.3 \pm 1.9$	$0.9 \pm 1.9$	0.02 (S)
	Placebo	61	$0.9 \pm 0.8$	$0.7 \pm 1.3$	0.28 (NS)
Urine ( $\mu\text{g/l}$ )	Selenium	60	$67.7 \pm 111.4$	$31.8 \pm 77.8$	0.00 (S)
	Placebo	64	$95.5 \pm 147.0$	$41.6 \pm 78.8$	0.00 (S)
<i>Concentration of selenium</i>					
Serum ( $\mu\text{g/l}$ )	Selenium	50	$95.7 \pm 52.2$	$115.8 \pm 79.8$	0.12 (NS)
	Placebo	56	$45.9 \pm 31.1$	$52.1 \pm 41.2$	0.39 (NS)

S= Significant; NS= Not significant

## **Discussion**

The study result showed that majority of the subjects was young adult with preponderance of female. They have been drinking shallow tube well water contaminated by arsenic for more than 11.6 years. But fortunately cooking water used was from ponds. Most of the subjects were from poor socio-economic groups and have large family.

The cardinal sign showed in the subjects were melanosis, leucomelanosis and keratosis. After intervention there were marked improvement of melanosis and keratosis. There was significant reduction in total arsenic load found in the subjects in nail, hair and urine after intervention and serum selenium concentration become rising.

Many researchers found that selenium is a known antioxidant and has an ability to negate the toxic effects of several heavy metals including arsenic. The interaction between arsenic and selenium was reported by Levander (1977) and concluded that arsenic has a protective effect against the toxicity of a variety of forms of selenium. The metabolic antagonism between arsenic and selenium as arsenite stimulated the excretion of selenium into the bile, so did selenite stimulate the excretion of arsenic (Levander, 1977). Antagonistic interactive effects were also observed between arsenic and selenium including complete to partial alleviation of the selenium toxicity (Hoffman, 1992). Moreover, both arsenic and selenium interact extensively with -SH groups in tissues, it is possible that arsenic elimination is delayed in selenium-deficiency state because there could be more target -SH groups for arsenic to react with because selenium is low (Kenyon, 1997). On the contrary, if sufficient selenium is available to the tissues, the -SH group would not be left free for arsenic to react with and there would be abundance of antioxidant enzymes to counteract the per-oxidative stress by arsenic (Passwater, 2000). A Chinese study (Hu, 1989) done among the workers exposed to arsenic showed selenium found antagonize the toxic effect of arsenic.

Population thriving on diets low in methionine is likely to suffer more from arsenic exposure. Research during the past quarter of century has identified many essential trace elements whose function were previously unknown, and that marginal or severe imbalances can be considered risk factors for several diseases of public health importance (Mertz, 1981).

In a study by Wuyi et al., 2001 that selenium can prevent the accumulation of arsenic in the human body and rectify the damages in the experiment.

After the administration of 100-200 µg selenium per day for 14 months, 75.0% and 55.0% of the patients served as patients for selenium-therapy group in clinical examination and symptom, and 25.6% and 24.4% as control group. In the selenium-therapy group, liver function, hepatic ultrasonography, electrocardiogram and electron microscope observation of erythrocyte reversed significantly than the control as 80%, 60%, 72.2%, 84.7 % versus 46.1%, 30.7%, 0%, 44.8% respectively. They found that oral selenium supplementation can effectively decrease arsenic concentration in hair, urine and blood of the selenium-group much more than that of control group and reverse the arsenic related skin lesions and symptoms (Wuyi, 2001)

G. Schrauzer concluded that ten-fold lower oral dosage of organic selenium produced 2-fold greater increase in selenium levels in the blood; organically bound selenium is at least 20-fold more effective in providing the body with the trace element. The form of selenomethionine that the body can use is l-selenomethionine which is better absorbed and incorporated into the body components than other forms. Studies in New Zealand and Finland showed that selenomethionine was at least 75% bioavailable compared to 59% for sodium selenite and raised blood selenium level higher, more rapidly and sustained longer than inorganic selenium (Valentine, 1994).

So, overall observations and results of our study indicate the significant beneficial effect of supplementation of selenium for 12 months in reducing the accumulation of arsenic after chronic exposure and we found no toxicity in study subjects.

## References

- Bangladesh Arsenic Control Society. Double-blind, randomized, placebo-controlled trial of antioxidant vitamins and minerals in the treatment of chronic arsenic poisoning in Bangladesh. BACS, Dhaka, 2003, pp 1-103.
- Biswas S, Talukder G, Sharma A. Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice in vivo. *Mutat Res.* 1999; 441: 155-60.
- Gregus Z, Gyurasics A, Csanaky I. Effects of arsenic-, platinum-, and gold-containing drugs on the disposition of exogenous selenium in rats. *Toxicol Sci.* 2000; 57: 22-31.
- Hoffman DJ, Sanderson CJ, LeCaptain LJ, Cromartie E, Pendleton GW. Interactive

- effects of arsenate, selenium and dietary protein on survival, growth and physiology in mallard ducklings. *Arch, Environ Contam Toxicol.* 1992; 22: 55-62.
- Hu GG. [Investigation of protective effect of selenium on genetic materials among workers exposed to arsenic]. *Zhonghua Yu Fang Yi Xue Za Zhi.* 1989; 23: 286-88.
- Kenyon EM, Hughes MF, Levander OA. Influence of dietary selenium on the disposition of arsenate in the female B6C3F1 mouse. *J Toxicol Environ Health.* 1997; 51: 279-99.
- Levander OA. Metabolic interrelationships between arsenic and selenium. *Environ Health Perspect.* 1977; 19: 159-64.
- Mertz W. The essential trace elements. *Science.* 1981; 213: 1332-8.
- Passwater RA. New discovery expand our knowledge about selenium's importance. 2000. In: Interviews with nutritional experts. <http://www.healthworld.com>.
- Rabbani GH, Saha SK, Akhtar M, Marni F, Mitra AK, Ahmed S, Alauddin M, Bhattacharjee M, Sultana S, Chowdhury AK. Antioxidants in detoxification of arsenic-induced oxidative injury in rabbits: preliminary results. *J Environ Sci Health Part A Toxicol Hazard Subst Environ Eng.* 2003; 38: 273-87.
- Schrauzer GN. Selenium: mechanistic aspects of anticarcinogenic action. *Bio Trace Elem Res.* 1992; 33: 51-62.
- Schrauzer GN. Selenomethionine: a review of its nutritional significance, metabolism and toxicity. *J Nutr.* 2000; 130: 1653-6.
- Schrauzer GN. The nutritional significance, metabolism and toxicology of selenomethionine. *Adv Food Nutr Res.* 2003; 47: 73-112.
- Spallholz, JE, Mallory Boylan, L and Rhaman, MM. Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Sci Total Environ* 2004; 323, 21-32.
- Styblo M, Serves SV, Cullen WR, Thomas DJ. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem Res Toxicol.* 1997; 10: 27-33.
- Styblo M, Thomas DJ. In vitro inhibition of glutathione reductase by arsenotri-glutathione. *Biochem. Pharmacol.* 1995; 49: 971-77.
- Styblo, M, Serves, SV, Cullen, WR and Thomas, DJ. Comparative inhibition of yeast glutathione reductase by arsenicals and arsenothiols. *Chem Res Toxicol.* 1997; 10: 27-33.

Valentine JL, Cebrian ME, Garcia-vargas GG, Faraji B, Kuo J, Gibb HJ, Lachenbruch PA. Daily selenium intake estimates for residents of arsenic-endemic areas. *Environ Res.* 1994; 64: 1-9.

Wuyi W, Linsheng Y, Shaofan H, Jian'an T, Hairong L. Prevention of endemic arsenism with selenium. *Curr Sci.* 2001; 81: 1215-18.