

The clinical Toxicology, 44: 1-7, 2006**11****Efficacy of Spirulina Extract plus zinc in Patients of chronic arsenic Poisoning: A Randomized Placebo-Controlled Study****Mir Misbahuddin**

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Background. Millions of people in Bangladesh, India, Taiwan, and Chile are consuming high concentration of arsenic through drinking water. And thousands of them have already developed chronic arsenic poisoning. There is no specific treatment. Some authors suggest the use of vitamins and minerals for more than 6 months. The present placebo-controlled double-blind study was conducted to evaluate effectiveness of spirulina extract plus zinc in the treatment of chronic arsenic poisoning. **Methods.** Forty-one patients of chronic arsenic poisoning were randomly treated orally by either placebo (17 patients) or spirulina extract (250 mg) plus zinc (2 mg) (24 patients) twice daily for 16 weeks. Each patient was supplied with arsenic-safe drinking water by installing a locally made water filter at household level. Effectiveness of spirulina extract plus zinc was evaluated by comparing changes in skin manifestations (clinical scores), arsenic contents in urine and hair, between the placebo- and spirulina extract plus zinc-treated group. **Results.** The concentrations of total arsenic in water (without filtration) of placebo- and spirulina extract plus zinc-treated groups were 150.1 ± 18.3 and 161.7 ± 23.9 $\mu\text{g/l}$, respectively. Intake of these high concentrations of arsenic

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lead to increased excretion of these high concentrations of arsenic lead to increased excretion of arsenic in urine ($72.1 \pm 14.5 \mu\text{g}/1$ in placebo-treated group and $78.4 \pm 19.1 \mu\text{g}/1$ in spirulina plus zinc-treated group). Sgyrt 2 weeks of using filtered water, there were significant reduction of both arsenic intake through water and

urinary arsenic excretion ($8.3 \pm 3.6 \mu\text{g}/1$ and $18.4 \pm 7.3 \mu\text{g}/1$ in placebo group; $9.7 \pm 5.4 \mu\text{g}/1$ and $21.6 \pm 5.8 \mu\text{g}/1$) in spirulina extract plus zinc treated group, there was a sharp increase in urinary excretion of arsenic ($138 \pm 43.6 \mu\text{g}/1$) at 4 weeks following spirulina plus zinc administration and the effect was continued for another 2 weeks. Spiraling extract plus zinc removed 47.1% arsenic from scalp hair. Spiraling extract had nomajor adverse effect that required physimian's attention. The clinical scores (median) for melanosis before and after treatment with placebo was not statistically significant ($p < 0.05$). Whereas in spirulina extract plus zinc-treated group it was statistically significant ($p < 0.01$) In cases of keratosis, the median clinical scores before and after treatment was not statistically ($p > 0.05$) in placebo-treated group. In spirulina extract plus zinc-treated group, the clinical score for keratosis before and after treatment was statistically significant ($p < 0.05$). Conclusions: Results show that spiraling extract (205mg) plus zinc (2mg) twice daily for 16 weeks may be useful for the treatment of chronic arsenic poisoning with melanosis and keratosis.

Keywords Chronic arsenic poisoning: Spirulina: zinc

Received 8 October 2004; accepted 8 June 2005

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BACKGROUND

In 1993. Bangladeshi people realized that they were exposed to high concentrations of inorganic arsenic (more than 50 ppb) through drinking water (1). Shallow tubewells are the main source of drinking water and surveys show that shallow tubewells in 59 out of 64 districts has been contaminated with various degrees of arsenic concentration. The Total population (57 million) is consuming arsenic (2). More than 30.000 people have already developed the signs and symptoms of chronic arsenic poisoning. Involvement of skin is the first manifestation, characterized by areas of hyper pigmentation on the upper chest and arms. keratosis develops later on at the palms and soles with fissures, cracks and warty lesions. Chronic arsenic exposure induces cardiovascular diseases, developmental abnormalities, neurologic and neurobehavioral disorders. diabetes. hearing loss. hematological disorders. and various types of eancer (3.4). Thus, the economy of the patient as well as the nation will be affected.

Still, there is no specific treatment. After diagnosis, the first step is to stop drinking arsenic contaminated water and allow the patient to use arsenic-safe water. Chelating agents, like dimercaprol (BAL),2-3dimercaptopropanesulphonate sodium (DMPS) and meso-2,3diercaptosuccinic acid (DMSA), are effective in the treatment of acute arsenic poisoning, but their usefulness is yet to be established in treating chronic arsenic poisoning (5.6) The beneficial effect of oral supplementation with retinol in the treatment of cutaneous chronic arsenic poisoning was first described more than 50 years ago (7). More recently. patients with cutaneous aresenicosis who were treated for 2 to 7 months with oral etretinate, a synthetic aromatic retinoid. showed clinical and histopathological improvement (8). Combination of vitamins A'E and C were used to treat the cases of chronic arsenic poisoning (9). Combination of several vitamins and minerals (beta-carotene, ascorbic acid, alphatocopherol, folic acid, zinc and selenium) were found to be effective in the removal of tissue arsenic by increasing its metabolism (10). the use of several antioxidants at a time needs rationality. At the same time, long duration of treatment up to 12 months may affect the compliance of the patient, in addition, there is a common belief among the patients that a vitamin is not adrug at all, Combined administration of vitamin C pus, DMSA, and vitamin E plus monoisoamyl.

DMSA in rats led to a more pronounced depletion of brain arsenic (11). Spirulina. a filamentous, unicellular alga, is a cyanobacterium growth in certain countries as food for human and animal consumption (12). It is a rich source of proteins. vitamins, amino acids, minerals and other nutrients. Spirulination. was found to be effective in the removal of arsenic from arsenic-loaded tissues in rats (13). Subsequently, administration of 10 g of spirulina per day for 3 months was reported

to be effective in the treatment of chronic arsenic poisoning (14-16). However, these studies were not scientifically sound, as no placebo treatment was given, The large dose of spirulina in powder or tablet form may affect the compliance of the patient, In addition, it has offensive smell and taste. Zinc is a known antioxidant and is shown to prevent acute arsenic toxicity in mice (17). Supplementation of Zinc also reduces the accumulated arsenic from different tissues of rat, following chronic exposure to arsenic (18).

In the present study we used spirulina extract plus zinc and conducted a double-blind placebo controlled trial for the treatment of chronic arsenic poisoning. Thus, the volume of drug will be decreased, offensive smell and taste will be overcome and thereby the compliance will be good.

SUBJECTS AND METHODS

The study was conducted during July 2003 to July 2004 with the approval of the ethical committee of the Bangladesh Medical Research Council (BMRC).

Study Area

The study was carried out at Muradnagar Upazilla (total area of 339 Sq. km) of Comilla District, which is about 110 km southeast of Dhaka, This upazilla is one of the high-density populated (total population is 577,971) areas. There are 30,199 tubewells, of which 93.5% are contaminated with a high concentration of arsenic (more than 50 µg/l). On average 19.14 persons are using the water of one tubewell for drinking and cooking purpose.

Patients

Patients with skin manifestations (suspected chronic poisoning) were requested by announcement to attend either the Muradnagar Health Complex, or temporary Arsenic Camp at Babutipara (within the Upazilla but 16 km away from the Muradnagar Health Complex), to receive drug treatment, Initially, more than 200 patients responded, of which, 117 patients were finally diagnosed as chronic arsenic poisoning (confirmed by clinical and laboratory findings). Due to limited funding, we randomly selected only 50 cases to participate in the trial.

Patients were, at first, diagnosed clinically based on the skin manifestations of melanosis and keratosis, A questionnaire was filled out, including information on the source of drinking water, duration of arsenic exposure, smoking habits, previous history of taking drugs, etc, Both males and females were included in this study, The age of the patients ranged from 18 to 58 years, Pregnant women and patients with hepatic or renal failure, were not included in this study, The history of exposure to arsenic, and presence of arsenic in urine and/or hair, confirmed the diagnosis.

Each patient visited the Health Complex of Arsenic Camp one every two weeks. Each time, one acid-washed 20 ml polyethylene container was given to each patient to collect midstream urine at the spot, and another 100 ml bottle to bring filtered water during the next visit, Hair samples were collected twice (before and after completion of treatment). About 0.5g of hair (a pencil thickness) was collected from less readily contaminated sites close to the occipital area of the scalp (new growth

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hair). Hair longer than 1 inch from the scalp was excluded, Hair was placed in a small plastic bag before analysis, Three ml of blood was drawn from the cubital vein and kept in a small glass vial for transfer to the laboratory, The purpose of the study was explained to each patient and requested, him/her to sign a consent form. All the patients agreed to be included in the trial.

Effectiveness of spirulina was evaluated by clinical improvement by comparing changes in skin manifestations (clinical scores) and arsenic contents in skin manifestations (clinical scores) and arsenic contents in urine and hair, between the placebo- and spirulina extract plus zinc treated groups, The total clinical score of the individual patient of manifestation was estimated by the presence of different degrees of melanosis (diffuse and rain drop). of keratosis (diffuse and spotted) in chest, abdomen, back, arms, palms, legs and soles, the degree of manifestation was marked as 0 for mild, 1 for mild, 2 for moderate and 3 for severe cases, Thus, the total score was the sum of marks of manifestation observed in different parts of an individual, the estimation of total score was done both before and after treatment to compare the effectiveness, Spot urine and hair samples were collected from 10 volunteers of an arsenic unexposed area (Dhaka City) for the estimation of total arsenic, They were randomly selected after announcement.

Water Filters for Arsenic Removal

Locally made water filters were distributed to the selected patients of chronic arsenic poisoning (one filter for each patient) immediately after confirming the diagnosis, to provide arsenic safe drinking water, Filters were manufactured from the ground having a single bucket with cover, iron chips and bricks were used to remove arsenic from arsenic-contaminated drinking water, Each filter was installed into the patient's house, Each patient was installed how to use the filter properly, and advised to use that water both for drinking and cooking purposes, to check the amount of arsenic present in the filtered water, each patient was advised to bring filtered water every two weeks, The amount of arsenic present in filtered water was measured and informed them at regular interval about their filtered water's arsenic level.

Manufacture of Caplets

At first, spirulina extract was prepared from spirulina flaked, spirulina flakes were then dried at room temperature and powdered by grinder, one at a time, approximately 5kg of spirulina powder was dissolved into 10 liters of 80% ethanol in a glass container, and kept for 24h. The liquid part of the suspension was separated by a filter 100 to 200 mesh, The solid part was again suspended in 5 liters of freshly prepared 80% ethanol for another 48 h. After filtration, all the ethanol extracts were combined and were filtered by 50 mesh filter paper and was collected in a 500 ml flask, evaporated in a rotary evaporator (40°C) Thus, green-colored alcohol extract of spirulina was obtained and washed twice with deionized water, It was finally freeze dried and kept at 0°C until the manufacture of caplets The whole extraction process was performed at room temperature with minimum exposure to light, About 2.0 kg of spirulina extract was prepared from 25 kg of spirulina flakes. This spirulina extract was then mixed with zinc and was supplied to a local pharmaceutical company to

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produce caplets. Each caplet contained 250 mg of spirulina extract and 2 mg of zinc. similar numbers of placebo caplet were prepared using glucose as inert substance, Both types of caplets had the same sizes, color (yellow colored film coating) and Smell. Ten caplets were packed in a blister. Packing in blister made the easy detection of compliance after observing the remnant of the pack following use of caplets by the patient. Only the code number was printed on each blister to identify its content. The disintegration time of each caplet was about 40 min.

Distribution of Drugs

After two weeks of using filtered water, patients were randomly selected from the list of patients for placebo and spirulina extract plus zinc-treated groups. Each patient received an envelope containing 28 caplets (drugs for 2 weeks). Each patient was advised to swallow one caplet twice daily (one in arsenic-safe drinking water immediately after eating and placing tick marks in the appropriate space printed on the envelope. The Total duration of treatment was 16 weeks. The patient was advised either to stop the drug immediately or to contact the local medical officer by cell phone for necessary measures if he/she felt any adverse effect (s). Each patient was also advised to keep his/her used blisters, and brought them during the next visit. The compliance of the patient was assessed by counting the number of caplets used and by examining the tick marks given on the envelope every day, During the study period patients were advised not to take any other antioxidant (s).

Laboratory Investigations

The amount of total arsenic in the drinking water and urine of 41 patients was estimated by SDDC (silver diethyldithio carbamate) spectrophotometric method. Pentavalent arsenic was reduced by potassium iodide. It was then reduced to arsine (AsH_3) by Zinc in

strong acid solution in an arsine generator. The arsine was then passed through a scrubber containing glass wool moistened with lead acetate, and into an absorber tube containing SDDC dissolved in chloroform, The arsine reacted with the silver salt, forming a soluble red color complex whose absorbance is read in a spectrophotometer. The maximum absorbance of the resulting color complex Was taken by a spectrophotometer (Shimadzu, Japan) at 525 nm. The limit of detection of arsenic in water and urine by this method was 7 $\mu\text{g/l}$. The hair samples for total arsenic were estimated by AAS with continuous flowing Hydride Generator (model 210 VGP, Buck Scientific Co, CT, USA). The hair was washed once with acetone and then sonicated in deionized water for 1 min in order to remove the externally bound arsenic. The hair was dried in an oven, weighed by an electronic balance and wet digested by acids (nitric acid, sulfuric acid and perchloric acid) for 2 h. The remaining volume (about 4 ml) waste constituted to 40 ml with water and an aliquot was analyzed by AAS. In brief (19): the sample, at first, digested with nitric acid, sulfuric acid and perchloric acid for 2 h by Bunsen burner. Following digestion, each sample was introduced into the Hydride Generator by continuous flow of 10% hydrochloric acid, 3% sulfuric acid and 1% sodium borohydride into a gas-liquid separator. The arsine vapor produced by arsenic and the hydrogen gas (produced by

sodium borohydride and acid) was carried by flowing argon gas into a quartz T-tube. [The tube was heated in an air-acetylene flame, to serve as an atomization cell.] The current of the Hollow Cathode Lamp for arsenic was 10mA. The wavelength and spectral bandwidth were 197.7 nm and 0.7 nm, respectively. The limit of detection was 0.1 µg/g of hair and the precision (within-day variations of replicate determinations) was 4.5%.

Quality Assurance Procedures

One physician examined all the patients every two weeks without seeing the patient's file. Another physician wrote the findings without informing the former physician about previous findings of the patient. Samples were collected using standard procedures, properly transported and preserved appropriately. Collected data at the field level were kept under strict confidence until analysis. Samples for laboratory investigations were coded. 5% of the total samples for arsenic estimation were validated by another reputed laboratory.

Statistical Analysis

We estimated the significance of difference of clinical scores between the before and after placebo or spiraling plus zinc-treated groups with the help of the Mann Whitney test, In the case of the placebo- treated group, the sample size was 17 and we estimated the value of T using

In the case of spiraling extract plus zinc-treated group, the sample size was 24 and we computed the data using

Other statistical analyses were carried out using standard software package (SPSS). t test was also used where necessary. Statistical significance was determined by $p < 0.05$.

RESULTS

Among the 50 patients, 9(8 from the placebo treated group and 1 from the spirulina-treated group) dropped out due to failure of taking drugs for more than 2 consecutive weeks (Fig.1). It may be due to no change in improvement of the clinical condition after taking drugs. Ultimately, 41 patients completed the intake of the drug, of which 14 were male and 27 were female.

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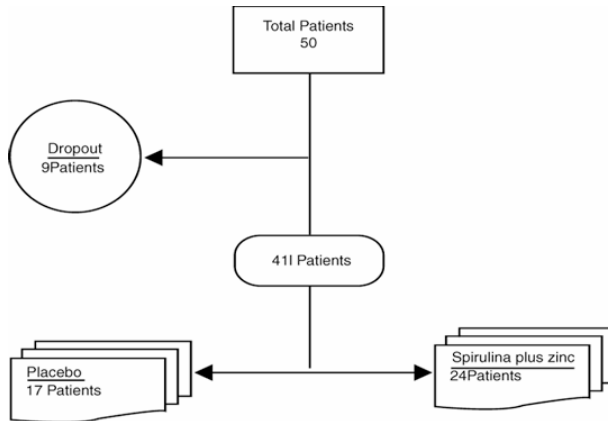


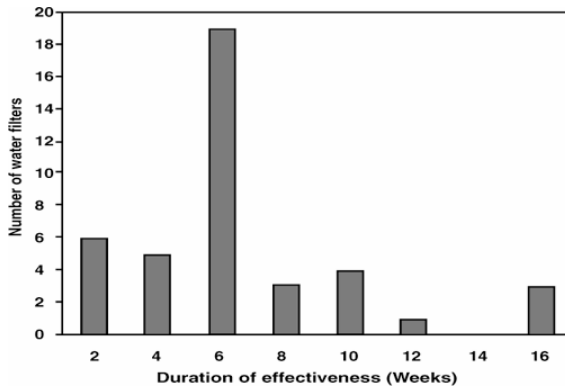
FIG. 1. Selection of Patients for clinical trial.

Among the male patients, 4 received placebo and 10 received spirulina extract plus zinc. Among the female patients, 13 received placebo and 14 received spirulina extract plus zinc. In total, 17 patients received placebo and 24 patients received spirulina extract plus zinc. The mean (\pm SD) age of placebo-treated group was 35.3 ± 8.1 years, whereas spirulina extract plus zinc-treated group was 32.1 ± 12.9 years. 91 % of the patients visited the Health Complex and Arsenic Camp at Babutipara personally in order to receive their drugs. Patients' compliance was evaluated. Intake of the drug by the patients was 98.2%. Fourteen patients (36.6%) were cigarette smokers, and none had a history of drinking alcohol. 73.2% patients had a previous history of taking vitamins or antioxidants for the treatment of chronic arsenic poisoning. The duration of taking shallow tubewell's water for drinking purpose was 20.3 ± 11.8 years. Among the 41 patients, 85.4% were using water from a tube well for drinking purpose. After the onset of chronic arsenic poisoning, only 14.6% patients changed their drinking water source from tubewell to either pond water or rain water.

The arsenic concentrations in tube well water before filtrations of placebo- and spirulina extract plus zinc-treated groups was 150.1 ± 18.3 and 161.7 ± 23.9 $\mu\text{g}/\text{l}$, respectively. The highest concentration of arsenic in tubewell water was 715 $\mu\text{g}/\text{l}$. The differences between these two groups were not statistically significant. The water filter was found to be effective to reduce the arsenic level of tube well water to 8.3 ± 3.6 , and 9.7 ± 5.4 $\mu\text{g}/\text{l}$ in placebo- and spirulina extract plus zinc-treated groups (94.5 and 94.4% removal of arsenic as examined after 2 weeks). But only 3 out of 41 filters (7.3%) provided arsenic safe drinking water throughout the study period (16 weeks; Fig. 2). The mean duration of effectiveness of the filters was 6.4 weeks.

The duration of suffering from symptoms was 4.8 ± 3.2 years. The highest percentage of skin manifestation (kurtosis) was found in soles (80.5%). Involvement of the palm was found in 78% of cases, Melanosis was present in chest, abdomen and 78% of cases. Melanosis was present in chest, abdomen and

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back as 68.3, 70.7 and 63.4% of cases, respectively. The maximum score for an individual was 13 for melanosis and 6 for keratosis. In cases of melanosis, the median clinical scores before and after treatment with placebo was not statistically significant ($p > 0.05$), whereas in spirulina extract plus zinc-treated group it was statistically significant ($p < 0.01$). In the case of keratosis, the median clinical scores before and after treatment was not statistically significant ($p > 0.05$) in the placebo-treated group. In spirulina extract plus zinc-treated group, it was statistically significant ($p < 0.05$). Apparent clinical improvements in both melanosis and keratosis were usually observed after 8-12 weeks,

Six patients experienced tinnitus of both ears immediately after starting drugs (Table 2). Among them, one was male and five were female, This complaint appeared immediately after starting the drugs and disappeared after 4 to 6 weeks. Other adverse effects were vertigo, pain in the lower abdomen and headache, The duration of these complaints were 2 to 8 weeks. The appearance of any adverse effect was not correlated with the intake of spirulins extract.

The amounts of arsenic in the urine of people not exposed to arsenic were not at detectable level, whereas patients of chronic arsenic poisoning contained arsenic 72.1 ± 14.5 , and 78.4 ± 19.1 $\mu\text{g}/1$ of urine in placebo- and spirulina extract-treated groups, respectively. After 2 weeks of using arsenic safe drinking water, the amounts of arsenic excretion in urine was reduced significantly (18.4 ± 7.3 $\mu\text{g}/1$ in placebo-treated group and 21.6 ± 5.8 $\mu\text{g}/1$ in spirulina extract-treated group). That is, there were 74.5 and 72.4% decreases in arsenic excretion in urine. Administration of spirulina extract plus zinc caused a sharp increase in urinary excretion of arsenic (138 ± 73.6 $\mu\text{g}/1$) at 4 weeks (Fig. 3; $p < 0.05$). These increased excretions of arsenic were not detected after 8 weeks, On the other hand, placebo did not show any increase in excretion of arsenic hand, placebo did not show any increase in excretion of arsenic in urine. The differences in arsenic urinary excretion between the two groups were not, at least, partially due to differences in renal clearance because the mean serum creatinin levels of both the groups were within normal range (data not shown).

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The amounts of arsenic in the hair of 10 individuals from arsenic non-exposed areas were estimated for comparison, the range of arsenic was 0.05 to 0.034 µg/g. The amount of arsenic in the hair of placebo- and spirulina extract plus zinc-treated patients before treatment were 3.08 ± 1.29 and 3.27 ± 1.16 µg/g, respectively (Fig.4). After 16 weeks treatment, arsenic contents were reduced to 2.99 ± 0.92 in placebo-treated group, and 1.73 ± 0.68 µg/g in spirulina extract plus zinc-treated group. Treatment with placebo did not show any significant change, but spirulina extract plus zinc removed 47.1% arsenic from hair, which was statistically significant ($p < 0.05$).

TABLE 1
Clinical scores of patients both before and after treatment using mann- Whitney test

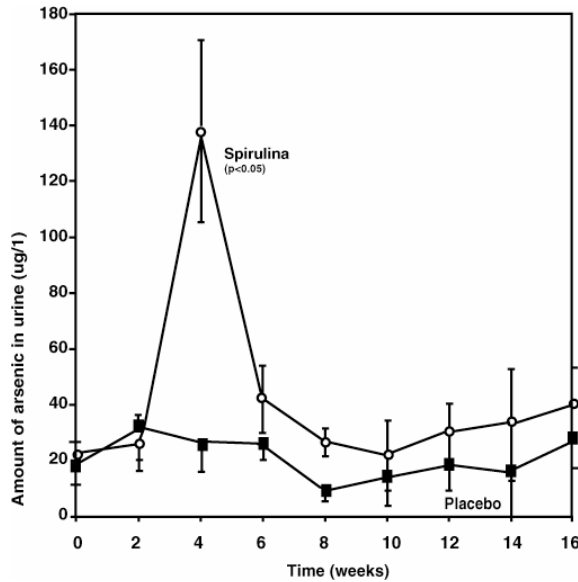
Patient	Patients	Clinical scores (median)		
		Calculated T or z value	Tabulated T or z value	p value
Melansosis Placebo	17	T=132.5	T=97	>0.05
Spirulina extract plus zinc	24	Z=3.26	Z=3.29	0.001<p<0.01
Keratosi Placebo	17	T=124.5	T=97	>0.05
Spirulina extract plus zinc	24	Z=2.26	Z.=2.58	0.01<p<0.05

TABLE 2
Adverse effects after administration of placebo and spirulina extract plus zinc containing caplets

	Number of cases	Drugs				Sex
		Placebo (n=17)	Spirulina extract Plus zinc (n=24)	Male	Female	
Tinnitus	6	4(23.5)	2(8.3)	1	5	
Vertigo	4	3(17.6)	1(4.2)	2	2	
Pain in lower abdomen	2	1(5.9)	1(4.2)	0	2	
Headache	1	0(0.0)	1(4.2)	0	1	

Data within parenthesis are percentage of patients from each group.

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FIB. 3. Urinary excretion of arsenic in spirulina extract plus zinc-treated parents from 0 to 16 weeks of treatment (○). Data of patients treated with placebo were shown for comparison (■). Data are mean \pm S.D. The excretion of arsenic in urine of spirulina treated patients at 4 h was statistically significant ($p < 0.05$) when compared the placebo group.

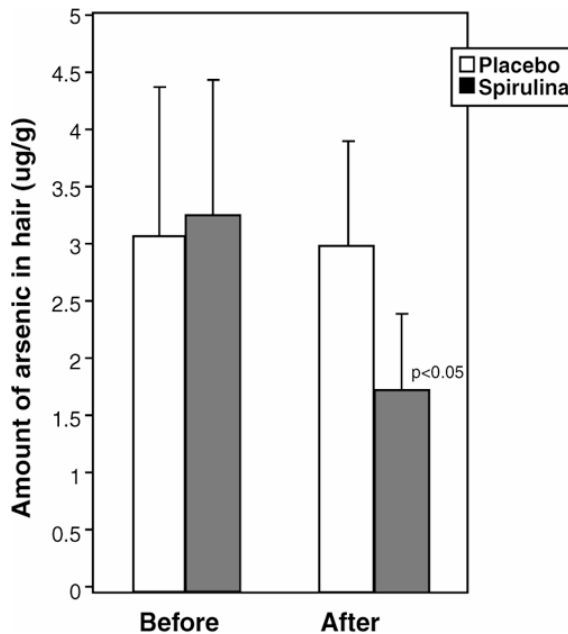
DISCUSSION

The present study shows that treatment with spirulina extract plus zinc for 16 weeks is effective for the clinical improvement of melanosis and keratosis. This treatment regime was found to be safe, as there was no major adverse effect that required physician's advice. Effectiveness of spirulina is due to enhanced removal of stored arsenic, as evidenced by decreased concentration of total arsenic in hair as well as increased excretion of total arsenic in urine. Intake of toxic levels of arsenic in drinking water did decrease reduced glutathione (GSH) levels in patients of chronic arsenic poisoning (20). Our study on rat shows that spirulina increased the level of GSH in arsenic-loaded liver tissues as well as enhanced the formation of DMA (Misbahuddin, unpublished data). Like spirulina, antioxidant vitamins are found to increase the urinary excretion of DMA and MMA (10). Spirulina is administered orally and its effects depend on the extent of absorption. Recently, it was found that chronic administration of high concentration of arsenic in rats inhibited the absorption and metabolism of a single dose of therapeutic concentration of paracetamol (21). This study may raise the need to examine the extent of absorption and metabolism of spirulina.

Chronic arsenic poisoning is due to chronic administration of high concentration of arsenic. Immediate stoppage of arsenic contaminated drinking water and the intake of arsenic safe drinking water are the precondition for the management of chronic arsenic poisoning. The people of arsenic-affected areas are, at present, fully

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dependent of the shallow tubewell as the source of drinking water. The present study shows that the water filter used did not provide arsenic safe drinking water throughout the study period of 16 weeks. The mean duration of effectiveness of the filters was 6.4 weeks. Rapid deterioration of the effectiveness of the filter may be due to high phosphate content in water of that area. Spirulina was effective even after short-term efficacy of the water-filtration system provided to the study participants. Maximal excretion of arsenic was observed at 4 weeks, but apparent clinical improvements in both melanosis and keratosis were usually observed after 8.12 weeks. The cause of this delay is not known, but it may be due to failure of providing arsenic-safe drinking water throughout the study period, However, spirulina, zinc of a combination would have an imract on the dermal findings that improved during therapy, without necessarily minimizing arsenic toxicity, but simply masking it. Simultaneous administration of spirulina and arsenic may reduce the effective of spirulina, but it does not cause any harmful effect, On the other hand, treatment with either selenium of zinc in presence of arsenic contaminated drinking water may increase the accumulation of arsenic in rats (22,23). So, the use of zinc in humans requires further study because it is very difficuly to provide arsenic-free drinking water while administering zinc,



ACKNOWLEDGEMENT

This work was supported by a grant from the World Health Organization, Bangladesh (project no. PHE BAN 003). We gratefully acknowledge the cooperation of participants in the study. We thank Mr. Han a. Heijanen, Dr. Deoraj

Caussy and prof. SK. Akhter Ahmad for their helpful comments and criticisms throughout the course of this study. We thank Prof. Md. Shahidullah for his help in statistical aspects.

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