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Effect of Spirulina on Biochemical Parameters and Reduction of Tissue Arsenic Concentration in Arsenic Induced Toxicities in Ducks

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Abstract: The present study was undertaken for the effect of spirulina on biochemical parameters and reduction of tissue arsenic concentration in arsenic induced toxicities in ducks. One hundred and seventy 5 ducklings were divided into five equal groups separately. One group (T_0) of ducklings was kept as control. One group (T_1) of ducklings were given arsenic trioxide @ 100 mg/L drinking water and rest three groups of ducklings $(T_2, T_3 \text{ and } T_4)$ were given arsenic trioxide @ 100 mg/L plus spirulina in three different doses i.e. 30, 60 and 120 mg/L in drinking water daily for 90 days starting from day 15. Five birds were sacrificed from each group in every 15 day intervals and biochemical parameters were determined. All the biochemical parameters (SGPT, SGOT, ALP, LDH and ACP) were significantly (p<0.01) elevated in arsenic treated groups. However, the elevation of these parameters was less in arsenic plus spirulina treated groups $(T_2, T_3 \text{ and } T_4)$. The distribution of arsenic concentration was highest in liver and lowest in faeces. Maximum reduction of arsenic was recorded in all organs following highest doses of spirulina (120 mg/L). The present study reveals that spirulina may be helpful for reducing the tissue burden of arsenic in ducks.

Key words: Spirulina, biochemical parameters, arsenic tissue concentration, arsenic toxicities, ducks

INTRODUCTION

In Bangladesh, nearly 62 out of 64 districts of the country's tube wells contain dangerous levels of inorganic arsenic, tube wells, which are serving as main sources for drinking and cooking purposes. The general populations are exposed to arsenic through drinking water, dust, fumes and dietary sources. The highest concentrations of arsenic were reported in seafood, rice, mushrooms and poultry in USA (Tao and Bolger, 1999). Roxarsone (3-nitro-4-hydroxyphenylarsonic acid), an organic arsenical compound is used widely in poultry production to control coccidial intestinal parasites. It is excreted unchanged in the manure and introduced into the environment when litter is applied to farmland as fertilizer. Although the toxicity of roxarsone is less than that of inorganic arsenic, roxarsone can be degraded biotically and abiotically, to produce more toxic inorganic forms of arsenic, such as arsenite and arsenate (Bednar et al., 2003). It has been reported that every U.S people may ingest 3.6-5.2 µg/inorganic arsenic daily from chicken alone consuming in an average 60 g chickens/day. Drinking water, dust, fumes and diet represent other forms of exposure. Inorganic forms of arsenic are classified as carcinogens, with chronic exposure (10-40 µg/day) associated with respiratory and bladder cancers (Lasky et al., 2004). Spirulina, microscopic blue-green algae, has a property of reducing heavy metals and nephrotoxic substance from the body. It is not only a whole food, but it seems to be an ideal therapeutic supplement. So far, no other natural food is found with such a combination and

amazing concentration of so many unusual nutrients like protein, β-carotene, amino acid, iron, βcarotene, phycocyanin, gama lenolenic acid, vitamin B₁, B₂, B₃, B₆, B₁₂, essential fatty acid etc. (Robert, 1989). β-carotene concentration of spirulina is 10 times higher than carrot. It was evident that food rich in β-carotene can reduce the risk of cancer (Peto *et al.*, 1981). It was found in the laboratory that the natural carotene and phycocyanin of spirulina also prevents cancer and its growth (Peto *et al.*, 1981; Shekelle *et al.*, 1981). In spirulina extract plus zinctreated group, the clinical scores for keratosis before and after treatment was statistically significant (p<0.05) (Misbahuddin *et al.*, 2006). The β-carotene in algae and leafy green vegetables has greater anti-oxidant effects than synthetic β-carotene (Amotz, 1987).

Ducks are one of the main source of meat in our Bangladesh. Duck meats may contain arsenic through arsenic contaminated water, growth promoters containing arsenicals and through arsenic medication. Arsenic is concentrated by many species of fish and shellfish and is used as a feed additive for poultry and livestock; fish and meat are therefore the main sources of dietary intake of almost 78.9%, according to a recent U.S. survey (Gartrell et al. 1986). In Canada, arsenic levels ranging from 0.4-118 mg/kg have been reported in marine fish sold for human consumption, whereas concentrations in meat and poultry range up to 0.44 mg/kg (Department of National Health and Welfare, Ottawa, 1983).

In the context of the above situation, the present study was undertaken with the following objectives:

- Study the effect of spirulina on biochemical parameters i.e., SGPT, SGOT, ALP, LDH and ACP in arsenic induced toxicities in ducks.
- Study the effect of spirulina on reduction of tissue arsenic concentration in arsenic induced toxicities in ducks

MATERIALS AND METHODS

The present study was undertaken to perform the effect of spirulina on biochemical parameters and reduction of tissue arsenic concentration in arsenic induced toxicities in ducks. The experiment was designed and following methodology was adopted for performing the experiment.

One hundred and seventy five, Xinding day old male ducklings were purchased from Kishoregonj Poultry Farm and fed with Aftab broiler starter feeds, Bangladesh. At fifteen day old the ducklings were randomly divided into 5 equal groups (n = 35) and were marked as group T_0 , T_1 , T_2 , T_3 and T_4 .

- T₀ = Ducklings were fed with recommended feed and drinking water *ad. lib*
- T₁ = Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg/L
- T₂ = Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L
- T₃ = Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L
- T₄ = Ducklings were fed with recommended feed and drinking water treated with arsenic trioxide@ 100 mg/litre plus spirulina@ 120 mg/L

Arsenic trioxide and spirulina at different dose rate were fed to different groups of ducklings with drinking water daily for 90 days. The parameters were taken fortnightly at day 15, 30, 45, 60, 75, 90 and 105. Fortnightly 5 birds were sacrificed from each group for studying following parameters:

- Biochemical parameters i.e., SGPT, SGOT, ALP, LDH and ACP
- Determination of arsenic in liver, heart, gastrointestinal tract, muscle, kidney, brain, bone and faeces in sacrificed birds at the end of the experiment.

Biochemical parameters: For determination of biochemical parameters blood samples were collected from five birds from each group at 15 days interval up to day 105 during sacrifice of the bird i.e., on day 15, 30, 45, 60, 75, 90 and 105. Total 2 mL of blood was collected in

the sterile glass test tubes. The blood containing tubes were placed in a slanting position at room temperature for 4 h. The tubes were incubated over night in the refrigerator. The serum samples were separated and centrifuged to get rid of unwanted blood cells. Serum samples were stored at -20°C for biochemical analysis. Serum Glutamate Pyruvate Transaminase (SGPT)/ALT, Glutamate Oxaloacetate Transaminase (SGOT)/AST and Alkaline Phosphatase (ALP) were determined by Reflotron® Plus (Boehringer Mannheim) according to the method described by Deneke and Rittersdorf (1984) and Deneke et al. (1985). Lactate Dehydrogenase (LDH) and Acid Phosphatase (ACP) were analyzed by using Microlab-300 spectrophotometer (Vital Scientific).

Determination of arsenic in different organs of sacrificed birds: The different tissues and organs (liver, heart, small intestine, thigh muscle, kidney, brain, femur and faeces) of 5 ducks from each group were collected on 105 days of age i.e. at the end of experiment for determination of arsenic and accordingly tissue homogenates were prepared and arsenic was determined by Hydride Generation Quartz Furnace Atomic Absorption Spectrophotometer as per method cited by Taggart et al. (2006).

Statistical analysis: The experimental data were designed in CRD and analyzed statistically using one way analysis of variance with the help of the MSTAT software and Duncan's Multiple Range Test (DMRT) were also done for ranging (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The results of the studies on the effect of spirulina on biochemical parameters and reduction of tissue arsenic concentration in arsenic induced toxicities in ducks following different doses are given below:

Biochemical parameters

Serum Glutamate Pyruvate Transaminase (SGPT):Daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water on SGPT in ducks is presented in Table 1.

The SGPT values increased 72.77% significantly (p<0.01) in arsenic trioxide fed group (T_1) in relation to control group (T_0). But the increasing percentage of SGPT values in other three groups (T_2 , T_3 and T_4) i.e., combined administration of arsenic and spirulina was 66.38, 56.75 and 42.27%, respectively, which was less than T_1 group.

Serum Glutamate Oxaloacetate Transaminase (SGOT): SGOT in ducks following daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days was estimated and presented in 2.

Table 1: SGPT/ALT (U/L) of control, arsenic induced and arsenic and spirulina treated ducks

	Days	Days	Days	Days	Days	Days	Days	Increased
Treatment	15	30	45	60	75	90	105	(%)
Control (T ₀)	5.24⁵	5.28°	5.31°	5.34°	5.38⁴	5.42⁴	5.45°	-
Arsenic trioxide@ 100 mg/L (T ₁)	5.17⁵	7.22°	9.47°	10.91ª	13.26°	16.43°	20.01 ^a	72.77
Arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L (T2)	5.34°	6.77⁵	8.42⁵	9.57⁰	11.21⁵	13.57⁵	16.21⁵	66.38
Arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L (T ₃)	5.19⁵	6.31°	7.24°	8.18°	9.29°	11.12°	12.60°	56.75
Arsenic trioxide@ 100 mg/L plus spirulina@ 120 mg/L (T ₄)	5.39°	5.82⁴	6.33⁴	6.72⁴	7.43⁴	8.77⁴	9.44⁴	42.27
SE	0.03	0.13	0.25	0.25	0.29	0.34	0.50	-

Figures indicate mean, SE (Standard Error); In a column figurers with same or without superscripts do not differ significantly as per DMRT; data were calculated at 99% level of significance (p<0.01)

Table 2: SGOT/AST (U/L) of control, arsenic induced and arsenic and spirulina treated ducks

	Days	Days	Days	Days	Days	Days	Days	Increased
Treatment	15	30	45	60	75	90	105	(%)
Control (T ₀)	172.86⁵°	174.68d	176.52°	179.74°	181.92°	184.86°	186.64°	-
Arsenic trioxide@ 100 mg/L (T ₁)	175.32*	181.44°	188.76°	196.24°	205.86	217.26	231.48	19.38
Arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L (T2)	171.62°	179.62ab	185.36⁵	192.02⁵	199.74b	207.92⁵	219.84b	15.11
Arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L (T ₃)	174.66ab	178.22⁵°	182.26°	188.16°	193.86°	199.82°	208.74°	10.59
Arsenic trioxide@ 100 mg/L plus spirulina@ 120 mg/L (T4)	173.38 ^{abo}	176.38°⁴	179.58d	184.64°	188.28⁴	192.14 ^d	198.06⁴	5.77
SE	0.67	0.78	0.63	1.14	1.11	1.43	1.35	-

Figures indicare mean, SE (Standard Error); In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01)

Similar to SGPT values, the SGOT values were also increased significantly (p<0.01) to the extent of 19.38% in arsenic trioxide treated group (T_1) in comparison to control group (T_0). However, the values were increased to a level of 15.11, 10.59 and 5.77% in T_2 , T_3 and T_4 groups, respectively, which were less than arsenic treated group.

Serum Alkaline Phosphatase (ALP): Result of daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days on ALP in ducks is presented in Table 3

The ALP values were Increased significantly (p<0.01) upto 20.20% in arsenic trioxide fed group (T_1) in relation to control group (T_0). But the increasing percent of ALP values in other 3 groups i.e. T_2 , T_3 and T_4 were 15.82, 11.22 and 6.74%, respectively, which were less than arsenic treated group.

Lactate Dehydrogenase (LDH): Serum LDH in ducks following daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days is recorded and presented in Table 4.

In ducks of arsenic treated group (T_1), the rise of LDH values was highest (13.98%) in comparison to control group (T_0). The rest three groups i.e. T_2 , T_3 and T_4 10.62, 7.30 and 4.13% given spirulina in 3 different doses along with arsenic trioxide.

Serum Acid Phosphatase (ACP): Result of daily administration of arsenic trioxide alone and in combination with spirulina in different doses in drinking water for 90 days on ACP in ducks is presented in Table 5.

The ACP values were increased significantly (p<0.01) upto 79.37% in arsenic trioxide fed group (T_1) in relation to control group (T_0) but in rest 3 groups i.e. T_2 , T_3 and T_4 , the percent (74.27, 65.66 and 48.84%) of increase on ACP values were somewhat low in comparison to T_1 group.

The serum levels of aminotransferases have been reported to be markedly elevated in animals exposed to arsenicals, the exact mechanism involved in elevation of these enzymes have not been conclusively postulated. Several workers have suggested that such effect may be the result of cellular damage (Drotman and Lawhorn, 1978) and/ or increased plasma membrane permeability (Ramazzotto and Carlin, 1978). In addition, factors such as increased synthesis or decreased enzyme degradation may also be involved (Dinman et al., 1963).

Ahmed (2004) observed significantly (p<0.01) increased values of SGPT and SGOT in arsenic treated rabbit. In a study, Chiou *et al.* (1999) found that SGPT, SGOT and ALP and LDH were increased in layers due to dietary arsenic. Similar to the present findings, elevation of biochemical parameters due to arsenic poisoning have been reported by many authors (Islam *et al.*, 2005; Sharma *et al.*, 2007; Olayemi *et al.*, 2002).

Islam *et al.* (2005) reported that SGPT and SGOT values were increased significantly (p<0.01) in arsenic treated rats. Similarly, Sharma *et al.* (2007) also observed that SGPT, SGOT, ALP and ACP were increased in arsenic treated group of Swiss albino mice.

In another study, Olayemi *et al.* (2002) studied on Plasma chemistry in 14 healthy adult (50-80 week old) and 10 healthy young (8-10 week old) Nigerian ducks (*Anas platyrhynchos*). They observed that young birds had significantly greater aspartate amino transferase (AST) and alanine amino transferase (ALT) values than the adult birds.

Table 3: ALP (U/L) of control, arsenic induced and arsenic and spirulina treated ducks

Treatment	Day 15	Days 30	Days 45	Days 60	Days 75	Days 90	Days 105	Increased (%)
Control (T ₀)	245.24 ^{ab}	248.88°	250.66⁴	253.34°	255.52°	258.18°	261.30°	-
Arsenic trioxide@ 100 mg/L (T₁)	247.82ª	255.34°	264.12ª	276.44°	291.64°	309.26°	327.44°	20.20
Arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L (T2)	241.46⁵	253.56 ^{ab}	260.62ab	270.26₺	282.36 ^b	296.22₺	310.38⁵	15.82
Arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L (T ₃)	248.62°	251.84™	257.32₺	264.58°	273.42°	283.76°	294.32°	11.22
Arsenic trioxide@ 100 mg/L plus spirulina@ 120 mg/L (T ₄)	243.38b	250.32 [™]	254.18⁰	259.24⁴	265.08d	272.94⁴	280.16⁴	6.74
SE	1.32	1.07	1.35	1.63	1.58	1.63	1.68	-

Figures indicate mean, SE (Standard Error); In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01)

Table 4: LDH (U/L) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	Increased
Treatment	15	30	45	60	75	90	105	(%)
Control (T ₀)	523.00	526.8 ^d	529.2°	533.2°	534.4°	536.8°	539.2°	-
Arsenic trioxide@ 100 mg/L (T₁)	525.20	539.2ª	553.8°	568.8ª	586.2ª	605.8	626.8ª	13.98
Arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L (T2)	521.80	535.8**	547.4⁵	559.2b	572.8 ^b	587.6⁵	603.2 ^b	10.62
Arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L (T ₃)	527.20	532.6⁵°	541.4°	549.4°	559.6°	570.8°	581.6°	7.30
Arsenic trioxide@ 100 mg/L plus spirulina@ 120 mg/L (T ₄)	524.80	529.2⁰⁴	335.8⁴	541.2⁴	547.4°	551.6⁴	562.4d	4.13
SE	1.32	1.41	1.42	1.67	1.60	1.57	1.75	-

Figures indicate mean, SE (Standard error); In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01)

In the present study, serum biochemical parameters were significantly elevated indicating some lesions or damages caused by arsenic trioxide. The rise of all parameters was maximum in T1 group (fed arsenic alone). The increase of these biochemical parameters were less in rest 3 groups (T2, T3 and T4) given spirulina in 3 different doses along with arsenic trioxide. It was also noticed that the rise of biochemical parameters minimum with higher dose of spirulina (120 mg/L) in drinking water indicating that spirulina has to some extent a protective role against arsenic induced tissue injuries. The exact cause of this protective role in recovering tissue damages is not fully understood. However, it is known that spirulina is an enriched source of nutrients like protein, amino acid, iron, β-carotene, phycocyanin, gama lenolenic acid, vitamin B₁, B₂, B₃, B₆, B₁₂, essential fatty acid etc which are very much helpful in maintaining the normal health. So, these findings indicate that spirulina has the positive role in decreasing the increased biochemical parameters due to arsenic toxicities.

Effect of spirulina on reduction of tissue concentration of arsenic: Arsenic concentration was determined in different tissues and faeces at 105 days following administration of arsenic trioxide alone and and in combination with spirulina and is presented in Table 6. After administration of spirulina at 3 different doses of 30, 60 and 120 mg/L in drinking water along with arsenic trioxide @ 100 mg/L to three groups ducks i.e. T_2 , T_3 and T_4 . The concentration of arsenic was gradually reduced in all organs. The decreasing trend of arsenic concentration was related to increase of spirulina dose i.e., the more of spirulina dose; the less concentration of arsenic was detected in all tested organs.

The highest percent of reduction of arsenic concentration in liver (79.07%), kidney (80.96%), thigh muscle (82.98%), small intestine (82.79%), heart (77.86%), femur (69.41%), brain (73.62%) and faeces (75.73%) was observed following highest dose of spirulina (120 mg/L). The highest percent of reduction upto 82.98% was observed in thigh muscle following highest dose 120 mg/L of spirulina along with arsenic trioxide.

In control group no arsenic was detected in any organs tested. However, following 100 mg/L of arsenic trioxide in T_1 group, the arsenic concentration was found highest in liver followed by kidney, small intestine, thigh muscle, heart, femur, brain and faeces.

Similar to present findings, many author observed highest concentration of arsenic in liver (Islam et al., 2001; Alam, 2004; Ahmed, 2004). Islam et al. (2001) observed the tissue distribution of arsenic in different organs with a maximum distribution in liver and kidney (upto 4.2 ppm) followed by spleen (upto 3.2 ppm), heart (upto 3.0 ppm) and dermis (upto 1.4 ppm) in arsenic fed rats. Alam (2004) found the tissue distribution of arsenic with highest cocentration in liver (3.0 ppm) followed by spleen (1.8 ppm), stomach (1.2 ppm), intestine (0.97 ppm), heart (0.86 ppm), muscle (0.8 ppm) and dermis (0.4 ppm) in arsenic induced rats. Likewise, Ahmed (2004) also reported that the tissue distribution of arsenic was highest in liver (1.2 ppm) followed by kidney (0.8 ppm), spleen (0.23 ppm), heart (0.4 ppm), muscle (0.08 ppm) and dermis (0.6 ppm) in arsenic treated rabbit. Pizzaro et al. (2004) and Taggart et al. (2006) studied on the distribution and biotransformation of arsenic species in chicken and duck cardiac and muscle tissues. Taggart et al. (2006) found that dry weight bone arsenic concentrations ranged from n.d -1.76 mg/kg and

Table 5: ACP (U/L) of control, arsenic induced and arsenic and spirulina treated ducks

	Day	Day	Day	Day	Day	Day	Day	Increased
Treatment	15	30	45	60	75	90	105	(%)
Control (T ₀)	2.60	2.76⁴	2.92⁴	3.08⁴	3.22⁴	3.36⁴	3.52⁴	-
Arsenic trioxide@ 100 mg/L (T ₁)	2.64	4.68°	6.62ª	8.84	11.28°	14.18 ^a	17.06°	79.37
Arsenic trioxide@ 100 mg/L plus spirulina@ 30 mg/L (T2)	2.72	4.18ab	5.68⁵	7.38⁵	9.08⁵	11.48⁵	13.68⁵	74.27
Arsenic trioxide@ 100 mg/L plus spirulina@ 60 mg/L (T ₃)	2.58	3.64⁵◦	4.76°	5.92°	7.06°	8.78°	10.28°	65.66
Arsenic trioxide@ 100 mg/L plus spirulina@ 120 mg/L (T ₄)	2.66	3.16⁰⁴	3.82d	4.46⁴	5.04⁴	6.08⁴	6.88⁴	48.84
SE	0.27	0.28	0.26	0.27	0.30	0.30	0.35	-

Figures indicate mean, SE (Standard error); In a column figurers with same or without superscripts do not differ significantly as per DMRT, data were calculated at 99% level of significance (p<0.01)

Table 6: Effect of feeding spirulina in different doses for 90 days on reduction of tissue concentration of arsenic (mg/kg WW) in ducks

			Thigh	Small				
Treatment	Liver	Kidney	muscle	intestine	Heart	Femur	Brain	Faeces
control (T ₀)	O _e	Oe	Oe	O ^e	Oe	O d	O _q	O _q
Arsenic trioxide@ 100 mg/L (T ₁)	1.778ª	1.692°	0.764°	1.116°	0.338°	0.17ª	0.0508ª	0.0338°
Arsenic trioxide@ 100 mg/L	1.292⁵	1.222b	0.538⁵	0.78b	0.268b	0.128ab	0.0368b	0.0254ab
plus spirulina@ 30 mg/L (T ₂)	(27.33)	(27.77)	(29.58)	(30.10)	(20.71)	(24.70)	(27.55)	(24.85)
Arsenic trioxide@ 100 mg/L	0.842⁰	0.748°	0.292⁰	0.428⁵	0.17⁵	0.09 ^{bc}	0.027 ^b	0.018 bc
plus spirulina@ 60 mg/L (T ₃)	(52.64)	(55.79)	(61.78)	(61.64)	(49.70)	(47.05)	(46.85)	(46.74)
Arsenic trioxide@ 100 mg/L	0.372 ^d	0.322^{d}	0.13 ^d	0.192 ^d	0.0748 ^d	0.052 ^{cd}	0.0134°	0.0082 ^{cd}
plus spirulina@ 120 mg/L (T ₄)	(79.07)	(80.96)	(82.98)	(82.79)	(77.86)	(69.41)	(73.62)	(75.73)
SE	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.00
Level of significance	**	**	**	**	**	**	**	**

Figures indicate the mean, SE (Standard error), **significant (p<0.01); In a column figurers with same or without superscripts do not differ significantly as per DMRT; Parenthesis indicates the reduction (%)

wet weight liver concentrations ranged from n.d -0.34 mg/kg arsenic in dabbling ducks.

In the present study, it was observed that spirulina could reduce the tissue burden of arsenic in all organs. Similar to present findings many other authors also reported that spirulina could reduce the tissue concentration of arsenic in many species (Robert, 1989; Misbahuddin *et al.*, 2006). Robert (1989) stated that spirulina; microscopic blue-green algae have the property of reducing heavy metals and nephrotoxic substance from the body. Misbahuddin *et al.* (2006) also observed that high concentrations of spirulina may lead to enhance the excretion of arsenic in urine.

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