Effects of spirulina in arsenic poisoning in the Black Bengal goat

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Abstract: Some of the pharmacological properties of spirulina (*Spirulina platensis*) may be linked to its antioxidant potential, which mitigates oxidative stresses. In this study we examined whether spirulina mitigated arsenic-induced toxicity in the Black Bengal goat (*Capra hircus*). Nonpregnant female goats of approximately 12 months old were used in noninduced, nontreated control (T_1); arsenic-induced, nontreated (T_2); and arsenic-induced, spirulina-treated (T_3) groups. Groups T_2 and T_3 were given sodium arsenite orally (5 mg/kg) daily for 15 weeks. For therapeutic justification of spirulina, 1 group of arsenic-treated goats (T_3) was given spirulina at 0, 50, 100, and 150 mg/kg daily for another 15 weeks. At the end of the experiment, the goats were sacrificed. The arsenic-treated goats showed the highest arsenic accumulation in the kidneys, followed by the liver, lungs, skin, muscles, and heart. Histological analysis demonstrated fibrosis and lymphocyte infiltration in the liver, degeneration of tubular epithelia and hemorrhage in the kidneys, and keratin deposition in the skin. IgG- and IgM-bearing lymphocytes were unaffected in arsenic toxicosis. Spirulina treatment did not show any significant therapeutic effects on arsenic toxicosis in goat. Therefore, spirulina may not be effective in treating arsenic toxicosis in small animals such as goats.

Key words: Arsenic, spirulina, Black Bengal goat, kidney

1. Introduction

Arsenic toxicity is a global health problem affecting millions of people. Arsenic is ubiquitous in the environment and is released from natural and man-made sources (1). At present, drinking water contamination by arsenic has been reported from at least 70 countries (2). As arsenic enters into the food chain, it is possible for a widespread distribution of arsenic throughout the plant and animal kingdoms. Goats that graze freely may be exposed to environmental pollutants like arsenic. Both organic and inorganic forms of arsenic exist in nature, and while goats are mainly exposed to inorganic arsenic through drinking water and feed, they are also exposed to some organic forms of arsenic through feed. The effects of arsenic accumulation after long-term intake of low doses to goats remain obscure. This may possess a potential dietary risk to humans (3), although little research has focused on goat meat as an additional source of arsenic exposure. Low-cost sustainable treatment of arsenic intoxication in goats is currently in demand.

The present knowledge on arsenicosis management in man and animals is inadequate. So far, there is no particular medical treatment that can either prevent or cure arsenicosis. Knowledge of arsenic metabolism can be useful in the management of arsenic toxicity in man and animals. Spirulina (*Spirulina platensis*), a blue-green alga, is rich in protein, phytonutrients, antioxidants, and polysaccharides that might have mitigating effects against heavy metal poisoning. Spirulina has been recommended as a chemoprotective against arsenic-induced toxicity in humans (4,5). It seems reasonable to expect that administration of spirulina might provide a protective mechanism against arsenic-induced toxicity in goats. Thus, the present work was undertaken to determine the effect of spirulina against arsenic-induced toxicity in Black Bengal goats (*Capra hircus*).

2. Materials and methods

Experiments were carried out at the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, Bangladesh. A completely randomized design was used in this work. All works were performed according to the ethics of laboratory animal use.

2.1. Animals

Twenty-four clinically healthy, nonpregnant Black Bengal female goats (*Capra hircus*) at the age of approximately 12 months and weighing 10–12 kg were used. They were housed in groups in a well-ventilated shed on wooden slats and were acclimatized to the environment for 2 months. Goats were dewormed by subcutaneous injection with nitroxynil (Nitronex, Renata Animal Health, Dhaka, Bangladesh) at 10 mg/kg and ivermectin (Acimec 1% vet, ACI Ltd., Dhaka, Bangladesh) at 0.2 mg/kg and were vaccinated against peste des petits ruminants (Livestock Research Institute, Dhaka, Bangladesh). Complete deworming was confirmed by evaluating fresh manure monthly by modified McMaster fecal egg counting test. Animals were kept on pasture daily between 0900 and 1600 hours and were supplemented with 200 g of concentrate daily along with ad libitum tube well water. Goats were grouped randomly into 3 treatment groups: noninduced, nontreated control (T_1, n = 4); arsenic-induced, nontreated (T_2, n = 4); and arsenic-induced, spirulina-treated (T_3, n = 16) groups. Subchronic arsenic toxicosis was developed in each goat of T_2 and T_3 by administering sodium arsenite (pro analysis grade, Merck, Germany) at 5 mg/kg daily for 15 weeks orally. T_1 goats were given 5 mL of distilled water as a vehicle daily for 15 weeks. At the end of 15 weeks, T_3 was further divided into 4 equal subgroups (T_{3-i}, T_{3-ii}, T_{3-iii}, and T_{3-iv}). Spirulina was fed to T_{3-ii}, T_{3-iii}, and T_{3-iv} at 50, 100, and 150 mg/kg daily, respectively, for another 15 weeks with gelatin and sucrose. Subgroup T_{3-i} being a positive control, received gelatin and sucrose daily as a placebo. Goats were observed daily for noticeable changes in feed and water consumption and signs of disease, and the condition(s) were recorded. At the end of the experiment the goats were euthanized with sodium pentobarbitone. Just after euthanasia, the liver, kidneys, lungs, thigh muscles, heart, skin (from neck region), femur, and prefemoral lymph nodes were harvested in physiological saline. For arsenic determination, aliquots of the liver, kidneys, lungs, thigh muscles, skin, heart, and femur were stored at –20 °C. For immunochemical analysis and histopathological examination, pieces of liver, kidney, skin, and prefemoral lymph node were fixed in 10% neutral buffered formalin.

2.2. Preparation of samples for detection of arsenic level by atomic absorption spectrophotometer

All reagents were of analytical grade. Millipore water was used throughout. Tissues were acid-digested in a block digester (M-24 plazas/samples, JP Selecta, Spain) according to procedures outlined by Cox (6) with minor modifications. Briefly, 1.5–2.5 g of tissue (wet wt., w/w) was taken in a digestion tube and digested by heating up to 150 °C, sequentially adding 5–7.5 mL of triple acid mixture (nitric acid - 10 parts, perchloric acid - 3 parts, and sulfuric acid - 1 part) and 3 mL of 30% hydrogen peroxide.

To validate the assay, 1 blank and 1 standard reference material (SRM) were prepared after every 15 samples. Concentrations of total arsenic (inorganic plus organic) in digested samples were determined using an atomic absorption spectrophotometer coupled with a hydride generator (PG Instruments Ltd., UK). The detection limit was 2 μg/L. Quantification of arsenic was performed by spiking samples with working standards of 0, 2.5, 5, 10, 15, and 20 μg/L, prepared immediately before use by serial dilution of the stock (arsenic pentoxide, Merck, Germany; 1,000,000 μg/L) in 10% hydrochloric acid. The salient features, instrument settings, and carriers were as follows: light source - ordinary hollow-cathode lamp; carrier gas - pure argon; carrier liquid - 1% hydrochloric acid; wavelength - 193.7 nm. Accuracy and precision of analyses were evaluated using commercially available SRMs from the National Institute of Standards and Technology (NIST), USA, with certified arsenic concentrations [NIST 1577b (bovine liver 47 ± 5 μg/kg, recovery rate 85%–103%)]. There was good conformity between obtained arsenic concentrations in the SRM and the reference values.

2.3. Histopathology

Formaldehyde-fixed tissues were processed through conventional histological procedures, cut into 5-μm-thick sections, and stained with routine hematoxylin and eosin (H&E). Immunostaining was performed as previously described (7) with few modifications. Briefly, prefemoral lymph nodes were cut into 5-μm sections from paraffin blocks and placed on silanized slides. Slides were deparaffinized and rehydrated, and antigen was unmasked using Tris/EDTA buffer (pH 9.0) at 95 °C for 4–5 min. The endogenous peroxidase activity was blocked by soaking the slides in 0.3% H_2O_2 in Tris-buffered saline (pH 7.4) for 15 min at room temperature. To reduce nonspecific background staining, slides were treated with 4% fetal calf serum at 37 °C for 45 min. Secondary antibody [for IgG - rabbit antigoat IgG labeled with HRP (Bangalore Genei, India), 1:1000 dilutions; for IgM - antigoat IgM (Rockland Immunochemicals, Gilbertsville, PA, USA), 1:100 dilutions] was applied and incubated at 37 °C for 45 min. Peroxidase conjugated affinity purified antirabbit IgG (H&L, Goat; Rockland Immunochemicals) at 1:1000 dilutions was also added for detecting IgM-bearing lymphocytes and reincubated at 37 °C for 45 min. A chromogen, 3-3'-diaminobenzidine tetrahydrochloride (DAB) (Bangalore Genei), was applied and incubated at room temperature in the dark for 10 min. Incubations were carried out in a humidified chamber to avoid drying the tissue. Between every step, slides were washed 3 times with PBS (pH 7.4) for 5 min each. In every batch, a negative control section was tested using PBS instead of a secondary antibody. Sections were counterstained with Harris's hematoxylin. Clear immunostaining under a light

microscope (600×) was interpreted as a positive staining result for IgG and IgM.

2.4. Statistical analysis

Data were analyzed using SPSS (SPSS Inc., Chicago, IL, USA). Treatment-related differences in arsenic residues in the tissues were determined using the independent samples t-test between 2 groups, while multiple comparisons of means were performed using the F-test with a significance level of $P < 0.05$ (8).

3. Results

3.1. Arsenic residue in tissues

The presence of arsenic (w/w) in the tissues were determined by atomic absorption spectrophotometer and is presented in the Table. Only a small amount of arsenic was found in tissues (w/w) of the control goats, with the highest distribution in the liver (186.3 ± 15.9 µg/kg). Fifteen weeks of successive sodium arsenite treatment resulted in significant ($P < 0.01$) elevations of several fold of arsenic (µg/kg) in all tissues compared to the control, with the highest distribution in the kidneys (2272.3 ± 100.3), followed by the liver (1726.0 ± 100.3), lungs (1309.3 ± 60.6), skin (1222.5 ± 94.5), thigh muscles (1093.3 ± 85.5), and heart (977.3 ± 62.7). Significant ($P < 0.01$) elevation of arsenic residue in bones (dry weight) of arsenic-treated goats (2486.0 ± 299.4 µg/kg) was observed as compared to the control (523.8 ± 59.9 µg/kg). At the end of spirulina supplementation, arsenic residues in tissues reverted to close to the pretreatment state, irrespective of employing spirulina. However, significantly ($P < 0.05$) greater arsenic in the bone of nonsupplemented arsenic-induced control and spirulina-supplemented goats were found as compared

to the control. Incorporation of spirulina did not show any significant ($P > 0.05$) effects on the diminution of arsenic from tissues.

3.2. Histopathological findings

Light microscopic examination of the livers of the control (T_1) goats did not show any pathology (Figure 1). The arsenic-treated (T_2) goats showed noncirrhotic portal fibrosis characterized by expansion of portal zones with streaky fibrous tissue proliferation, lymphocytic infiltration at the periphery of portal zone, and, in a few cases endothelial cell degeneration. Increased pyknotic nuclei of hepatocytes and drop-out necrosis were visible in focal areas and there was formation of syncytia. Nonsupplemented arsenic-induced control and spirulina-supplemented goats showed expanded portal zones with portal fibrosis, but there were fewer pyknotic nuclei in hepatocytes and lymphocyte infiltration.

We then examined tissues of the kidneys, vital organs of the body, to determine whether they were affected by exposure to arsenic poisoning. The control goats (T_1) did not display any histopathological changes (Figure 2). Arsenic-treated goats (T_2) showed mild to severe necrosis and degenerative changes. Hydropic degeneration of tubular epithelia and especially of proximal tubules, proteinaceous casts as evidenced by pink color masses in the tubules, and pyknotic nuclei of the tubular epithelia in arsenic-treated goats (T_2) were noticed. The epithelial cells around the renal blood vessels showed necrosis and damage, indicated by the presence of fibrosis replacing the damaged areas. Bowman's capsular spaces were expanded due to the shrinkage of the glomerular cells. There were profuse hemorrhages in the medulla, indicative of

Table. Residual concentration of arsenic in wet tissue following 15 weeks of oral administration of sodium arsenite (5 mg/kg) or spirulina in sodium arsenite-treated goats. Values are means ± SEMs.

Organ/tissues	Arsenic concentration (µg/kg)					
	Sodium arsenite treatment		Spirulina supplementation [†]			
	Control goats	Arsenic-induced goats	Nonsupplemented goats	Supplemented goats		
				50 mg/kg	100 mg/kg	150 mg/kg
Liver	186.3 ± 15.9	1726.0 ± 100.3[**]	139.0 ± 19.8	145.5 ± 16.7	135.0 ± 21.3	139.8 ± 28.2
Kidneys	126.3 ± 9.5	2272.3 ± 100.3[**]	161.0 ± 18.8	165.5 ± 32.5	125.3 ± 36.9	147.8 ± 22.3
Lungs	124.3 ± 15.5	1309.3 ± 60.6[**]	116.8 ± 22.9	109.0 ± 26.4	122.8 ± 27.9	108.8 ± 22.2
Heart muscles	106.5 ± 6.3	977.3 ± 62.7[**]	101.5 ± 19.1	103.5 ± 26.8	120.3 ± 24.0	102.0 ± 19.3
Thigh muscles	118.3 ± 9.7	1093.3 ± 85.5[**]	136.3 ± 5.2[ac]	151.0 ± 30.1[ac]	192.3 ± 30.6[a]	114.5 ± 15.0[bc]
Skin	125.0 ± 7.4	1222.5 ± 94.5[**]	132.5 ± 16.8	139.8 ± 19.6	148.5 ± 19.9	133.0 ± 7.2
Bones [‡]	523.8 ± 59.9	2486.0 ± 299.4[**]	842.3 ± 71.7	992.3 ± 78.7	993.8 ± 36.2	893.0 ± 132.0

[**]: Significant difference ($P < 0.01$) between control and arsenic-treated groups.
[†]: Any 2 means in a row having different superscripted letters differ significantly at a 5% level of probability after spirulina supplementation.
[‡]: Values in dry-weight basis.
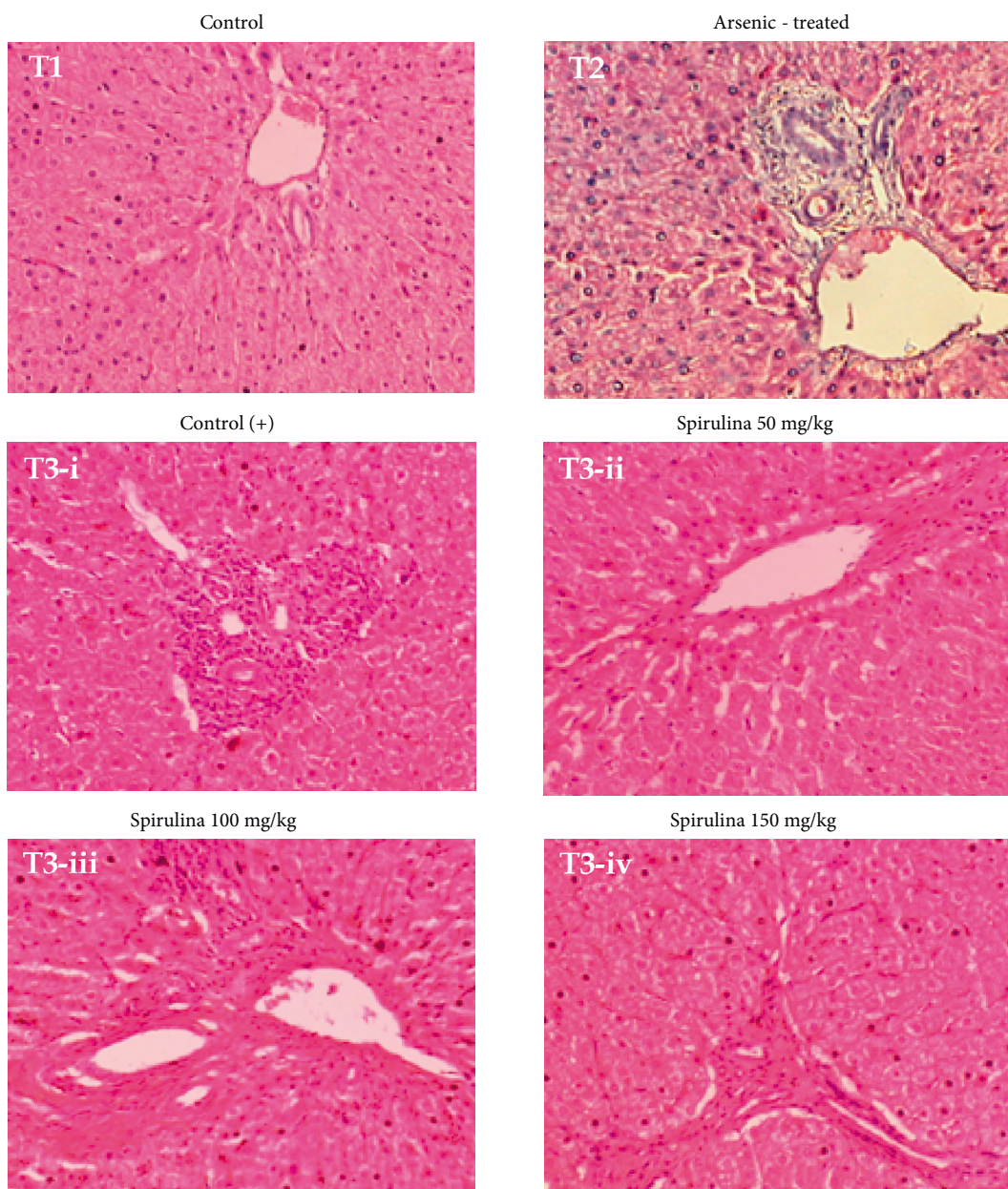
Histopathology of Liver

Figure 1. Histopathology of goat liver. T_1: Control, showing no change in hepatocytes. T_2: Sodium arsenite-treated (5 mg/kg orally daily for 15 weeks); pathological changes showing expansion of portal zones with streaky fibrosis, periportal lymphocytic infiltration, and endothelial cell degeneration. After 15 weeks, arsenic was terminated and the arsenic-treated animals were supplemented with spirulina at different doses (15 weeks), where 1 group was kept as the positive control (without spirulina). T_{3-I}: Control (+); $T3_{-ii}$: spirulina at 50 mg/kg; T_{3-iii}: spirulina at 100 mg/kg; T_{3-iv}: spirulina at 150 mg/kg; histopathological lesions in control (+) and all spirulina-fed groups depicting expansion of portal zones with portal fibrosis, lesser than in T_2 (H&E, 100× for all panels).

glomerular damage. In nonsupplemented arsenic-induced control and spirulina-supplemented goats (T_{3-ii}, T_{3-iii}, and T_{3-iv}), degenerative lesions of the convoluted tubules were improved, indicated by regular cellular arrangement,

absence of proteinaceous casts in the tubules, and few pyknotic nuclei of the tubular epithelia. Bowman's space recovered, but in a few cases, glomerular cells proliferated. The recovery was better in spirulina-supplemented groups
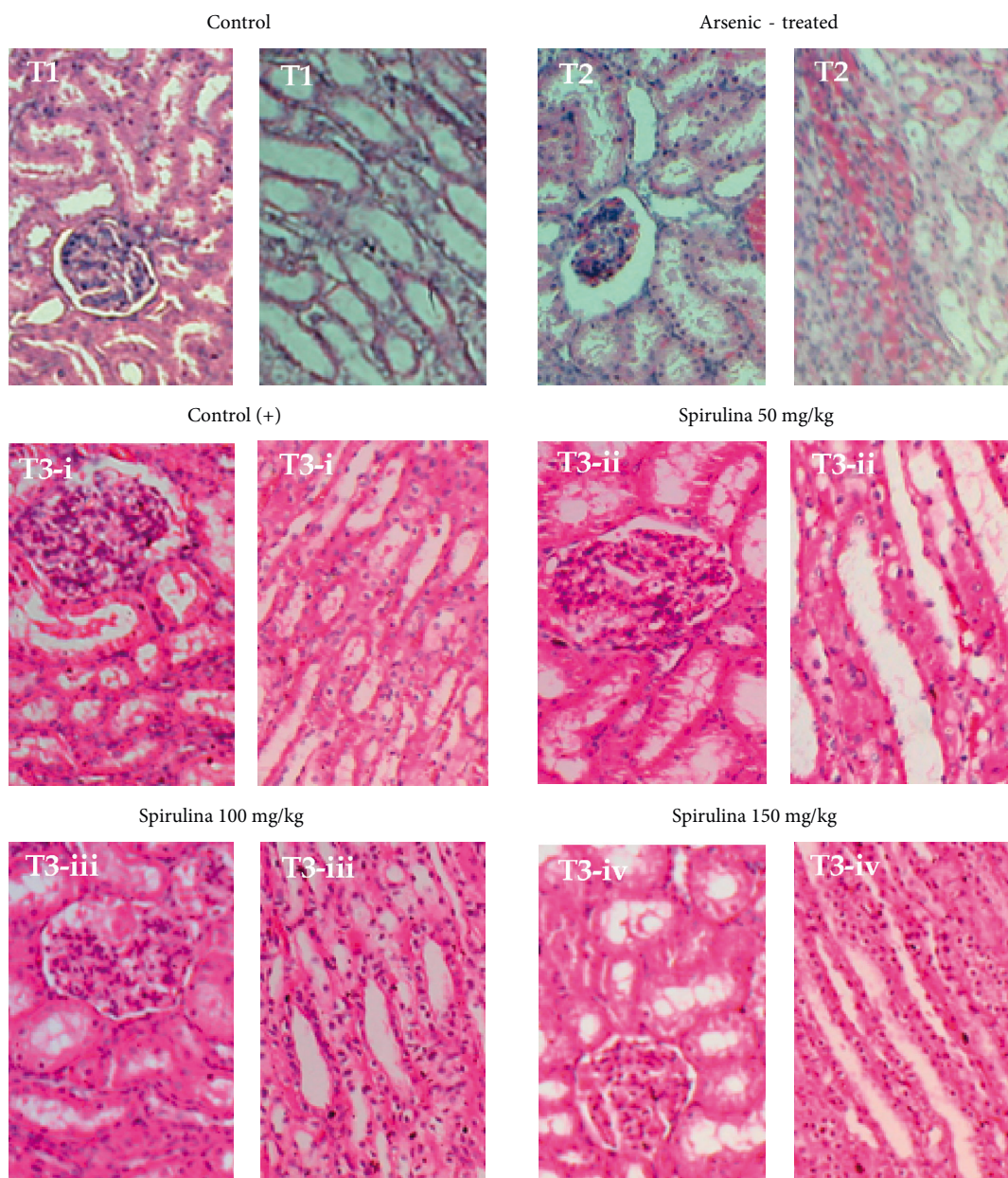
Histopathology of Kidney

Control

Arsenic - treated

Control (+)

Spirulina 50 mg/kg

Spirulina 100 mg/kg

Spirulina 150 mg/kg

Figure 2. Histopathology of goat kidney. T_1: Control, showing normal Bowman`s capsule and kidney tubules. $T_2$: Sodium arsenite-treated (5 mg/kg orally daily for 15 weeks); pathological changes showing hydropic degeneration of tubular epithelia, expanded Bowman`s capsular space, and hemorrhage in the medulla. After 15 weeks, arsenic was terminated and the arsenic-treated animals were supplemented with spirulina at different doses (15 weeks), where 1 group was kept as the positive control (without spirulina). T_{3-1}: Control (+); T_{3-ii}: spirulina at 50 mg/kg; T_{3-iii}: spirulina at 100 mg/kg; T_{3-iv}: spirulina at 150 mg/kg; histopathological lesions in control (+) and all spirulina-fed groups depicting few pyknotic nuclei of tubules and proliferated glomerular cells (H&E, 100× for all panels).

as compared to the nonsupplemented arsenic-induced control.

The skin is also a target organ for arsenic deposition, and changing of the skin structure would be an interesting finding in this study. Our histopathological analysis

is shown in Figure 3. Skin of the control goats was normal. In arsenic-treated (T_2) goats, mild thickening of the cornified layer of the epidermis was observed. The papillary cells proliferated, giving increased numbers of papillary fibroblasts. Mild infiltration of lymphocytes
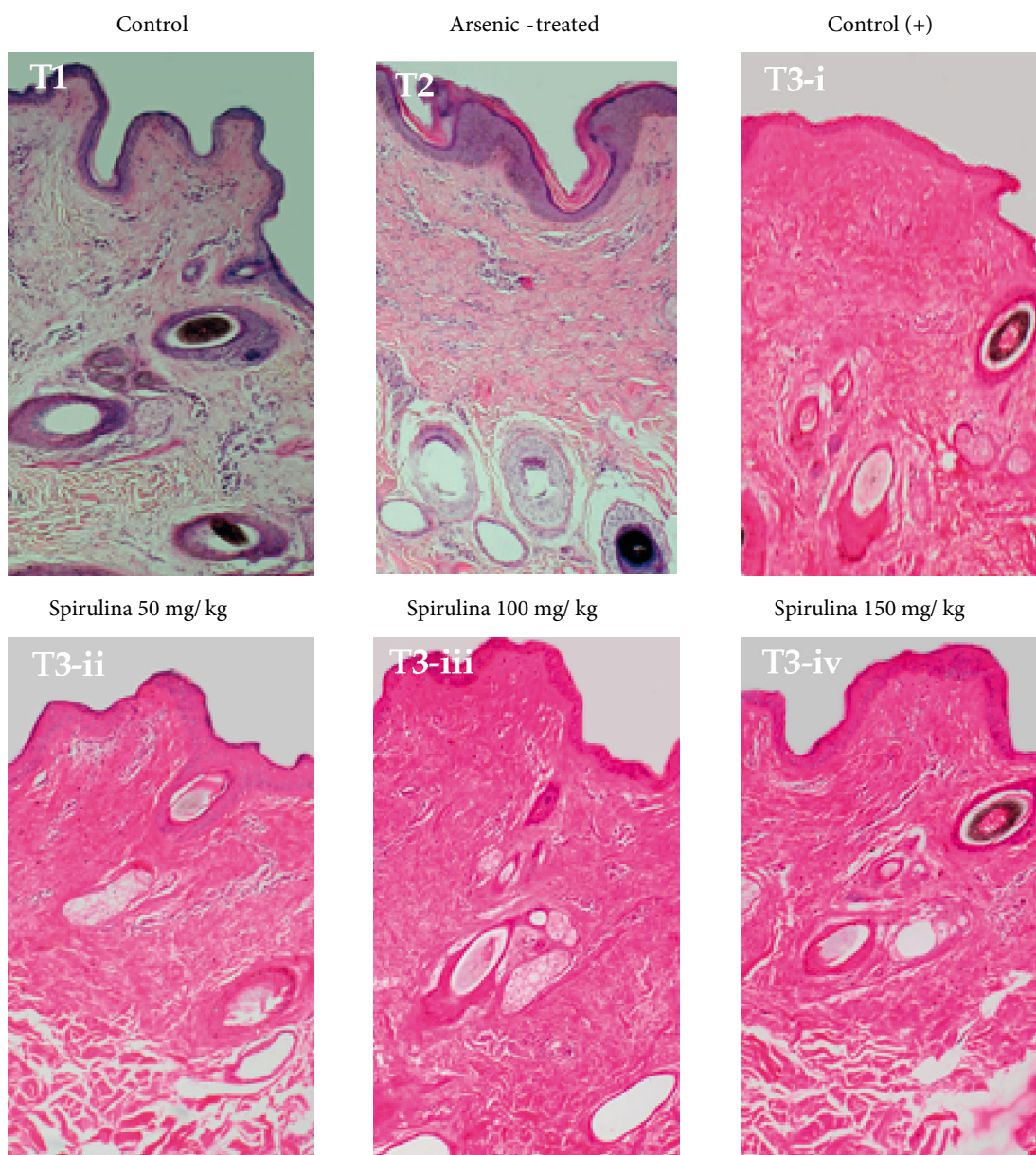
Histopathology of Skin

Figure 3. Histopathology of goat skin. T_1: Control, showing thin keratin layer with normal hair follicles. T_2: Sodium arsenite-treated (5 mg/kg orally daily for 15 weeks); pathological changes showing mild thickening of the cornified layer of the epidermis, proliferated papillary cells, and cystic appearance of the follicles. After 15 weeks, arsenic was terminated and the arsenic-treated animals were supplemented with spirulina at different doses (15 weeks), where 1 group was kept as the positive control (without spirulina). $T_{3\text{-}i}$: Control (+); $T_{3\text{-}ii}$: spirulina at 50 mg/kg; $T_{3\text{-}iii}$: spirulina at 100 mg/kg; $T_{3\text{-}iv}$: spirulina at 150 mg/kg; histopathological lesions in control (+) and all spirulina-fed groups depicting gentle proliferation of follicular cells and few cystic appearances of follicles (H&E, 100× for all panels).

and neutrophils was observed in the papillary areas. Numbers of follicular cells were increased. Some of the follicles were atrophied and there was destruction of some follicles, giving cystic appearance. Fifteen weeks after arsenic withdrawal, the histopathological sections of nonsupplemented arsenic-induced control and spirulina-supplemented goats revealed mild thickening of the keratin layer (stratum corneum) and mild proliferation of follicular cells. Cystic appearances of hair follicles, as in the arsenic-treated (T_2) group, remained constant.

Accumulations of a few lymphocytes were observed in the papillary layer of the dermis. No difference was observed in histopathological sections of skin between nonsupplemented arsenic-induced control and spirulina-supplemented goats or within spirulina-supplemented groups. The cutaneous manifestations of chronic arsenic poisoning have an insidious onset.

3.3. Immunohistochemical evaluations of IgG- and IgM-bearing lymphocytes

Any poisoning, whether subacute or chronic exposure, may have some influences directly or indirectly on the immune system of the body. Lymphocytes are an indication of the body's immune system in general phenomena. We investigated whether arsenic exposure in goats influences the immune system. Sodium arsenite treatment to the goats did not significantly alter the IgG-bearing lymphocytes in the lymph nodes compared to the control (Figure 4). The control goats showed 86.0 ± 3.0% IgG-positive lymphocytes, whereas the corresponding value was 81.8 ± 1.1% for arsenic-treated (T_2) goats. Supplementation of spirulina to arsenic-treated goats did not significantly (P > 0.05) modify the IgG-positive to IgG-negative lymphocyte ratio. IgM staining of lymph nodes showed 15.7 ± 1.5% positive lymphocytes in the control and 15.0 ± 0.5% in arsenic-treated (T_2) goats (Figure 5). Spirulina supplementation did not alter the IgM-positive lymphocytes in the lymph node. Arsenic has been recognized as a powerful immunomodulatory agent in many laboratory animals and in epidemiological studies.

4. Discussion

Our atomic absorption spectrophotometric evaluation showed low amounts of arsenic presence in the tissues of the control goats. However, 15 weeks of successive sodium arsenite treatment resulted in significant elevation of arsenic in all tissues as compared with the noninduced control. At the end of spirulina supplementation, arsenic residues in tissues reverted to close to the pretreatment state. It is to be mentioned that arsenic-induced nontreated control goats also reverted to close to the pretreatment state. Moreover, both supplemented and nonsupplemented spirulina showed significantly greater arsenic residue in the bone tissues. Goats did not show any clinical signs of arsenic toxicity. This may be due to the small dose and/or short duration of treatment. Arsenic is a slow poison and symptoms of arsenic poisoning develop only after prolonged consumption. The presence of arsenic in tissues of the control goats may be due to the consumption of food and water contaminated with traces of this element, which is universally distributed in Bangladesh (9). Accumulation of high arsenic residues in tissues of arsenic-treated goats may be due to repeated doses. Different concentrations of arsenic in different tissues indicate different distributions

(10). The highest arsenic residue in the kidneys of arsenic-treated goats is consistent with earlier findings (11). These metabolites and unchanged arsenic might accumulate in kidney tissues. Depletion of arsenic residue in tissues of nonsupplemented arsenic-induced control goats to the pretreatment state indicates self-detoxification by goats, or antioxidants in green grass may, at least in part, help to eliminate arsenic. However, the antioxidant role of green grass has not been proven to warrant further study. Higher arsenic residues in bone indicate its slower elimination from bone than other soft tissues.

Light microscopic examination of the livers of the control goats did not show any pathology. The arsenic-treated goats showed noncirrhotic portal fibrosis characterized by expansion of portal zones with streaky fibrous tissue proliferation. Periportal lymphocytic infiltration and endothelial cell degeneration were visible in a few cases. Both spirulina-supplemented and nonsupplemented groups showed expanded portal zones with portal fibrosis, but there were fewer pyknotic nuclei in hepatocytes and less periportal lymphocytic infiltration. The liver appeared to be a major organ of arsenic toxicity, possibly because of its unique vasculature as well as generation of potentially toxic metabolites. Chronic administration of inorganic arsenic to experimental animals produced various liver lesions in a dose-dependent manner, including inflammation and oxidative damage (12), ballooning of hepatocytes with lymphocytic infiltration in the periportal as well as parenchymal area, and drop-out necrosis. Increased pyknotic nuclei of hepatocytes in arsenic-treated goats are an indication of cellular necrosis. Arsenic exerts oxidative stress that results in depletion of the cellular endogenous antioxidant reserve, which leads to apoptosis (13). Due to its lipophilic nature, arsenic attaches to lipid, inducing lipid peroxidation that causes the deposition of lipid droplets in tissues; little or no such change may be due to low-fat diet and/or short duration of the experiment. Endothelial cell degeneration may be a consequence of direct effects of arsenic-induced inflammation on the vascular cells and indirect effects on the liver vasculature.

The kidneys of the control goats did not display any histopathological changes. Arsenic-treated goats showed mild to severe necrosis and degenerative changes. Hydropic degeneration of tubular epithelia and especially of proximal tubules, proteinaceous casts as evidenced by pink color masses in the tubules, and pyknotic nuclei of the tubular epithelia in arsenic-treated goats were noticed. The epithelial cells around the renal blood vessels were necrotized and damaged, as indicated by the presence of fibrosis replacing the damaged areas. Bowman's capsular spaces were expanded due to the shrinkage of the glomerular cells. There were profuse hemorrhages in

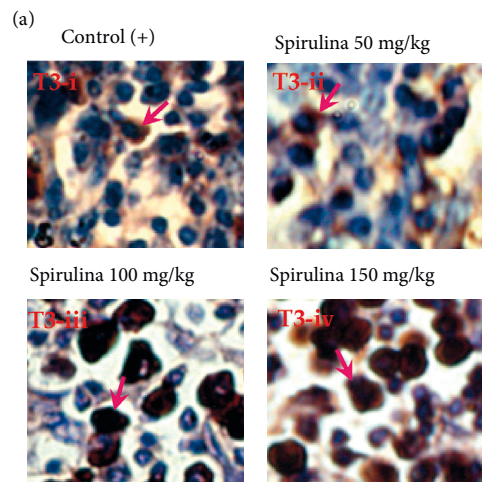Histopathology of Lymph node for IgG lymphocyte
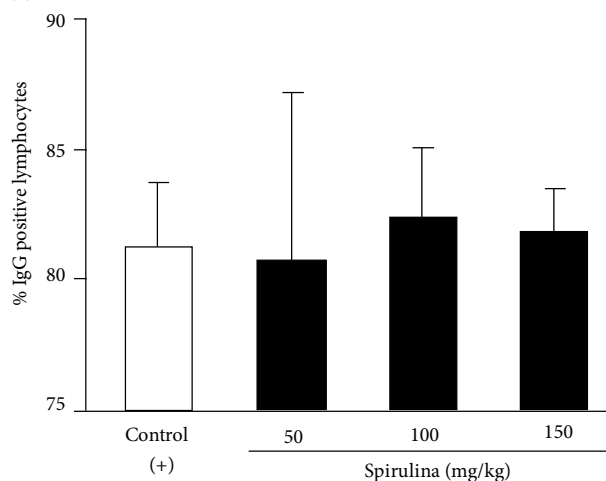
A

(a)

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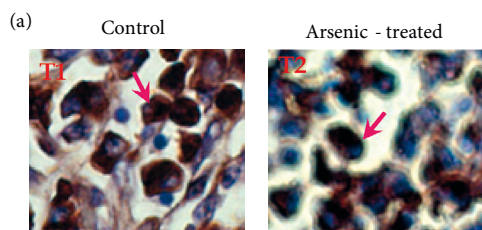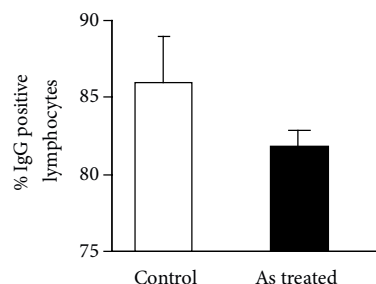
(b)

Figure 4. Immunohistochemical evaluation of IgG-bearing lymphocytes in the lymph nodes of goats. Animals were subjected to sodium arsenite treatment (5 mg/kg orally daily for 15 weeks). A(a): Immunohistochemical analysis photographs, T_1, untreated control, and T_2, sodium arsenite-treated. Arrow indicates IgG-positive lymphocytes (600× for both panels). A(b): Graphical representation of percentage (mean + SEM) of IgG-bearing lymphocytes in each group (n = 3 for each group). B: After 15 weeks of exposure, arsenic was stopped and the animals were supplemented with spirulina for another 15 weeks. B(a): T_{3-i}, control (+) (without spirulina); T_{3-ii}, T_{3-iii}, and T_{3-iv} were treated with 50 mg, 100 mg, and 150 mg spirulina/kg body weight, respectively. Arrow indicates IgG-positive lymphocytes (600× for all panels). B(b): Graphical representation of percentage (mean + SEM) of IgG-bearing lymphocytes in each group (n = 3 for each group).

the medulla, indicative of glomerular damage. Both in spirulina-supplemented and nonsupplemented groups, degenerative lesions of the convoluted tubules were improved, indicated by regular cellular arrangement, absence of proteinaceous casts in the tubules, and few pyknotic nuclei of the tubular epithelia. Bowman's space recovered, but in a few cases, glomerular cells proliferated. The recovery was better in spirulina-supplemented groups than the nonsupplemented group. The urinary system and especially the kidneys and bladder are considered the second target organs of arsenic toxicity. Bowman's capsule and the epithelial cells of proximal convoluted tubules of the kidneys are sensitive to arsenic (14,15).

In arsenic-treated goats, mild thickening of the cornified layer of the epidermis was observed. The papillary cells proliferated, giving an increased number of papillary fibroblast. Mild infiltration of lymphocytes and neutrophils were observed in the papillary areas. Numbers of follicular cells were increased. Some of the follicles were atrophied and there was destruction of some follicles, giving a cystic appearance. Fifteen weeks after arsenic withdrawal, the histopathology of spirulina-supplemented and nonsupplemented groups revealed mild thickening of the keratin layer (stratum corneum) and mild proliferation of follicular cells. Cystic appearances of hair follicles, as in the arsenic-treated group, remained constant. Accumulations of a few lymphocytes were observed in the papillary layer of the dermis. No difference was observed in the histopathology sections of skin between spirulina-supplemented and nonsupplemented groups or

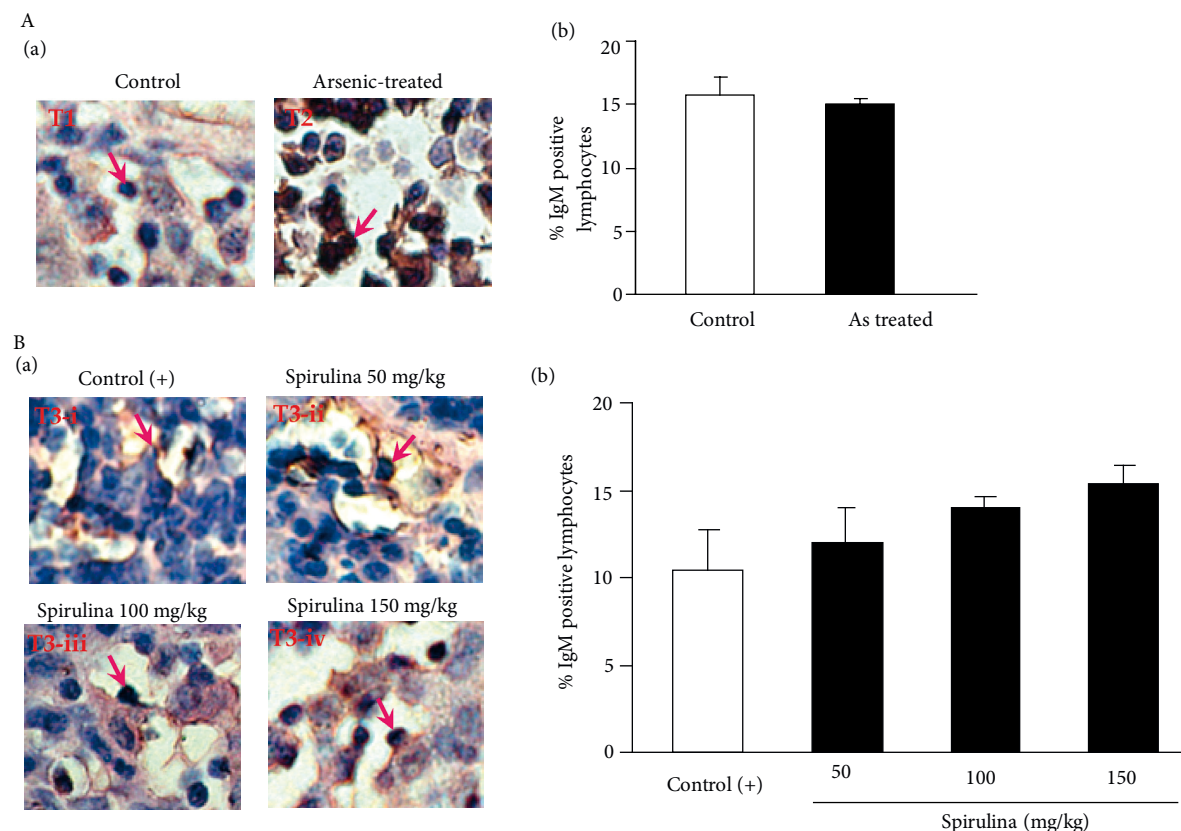Histopathological analysis of Lymph node for IgM lymphocyte

Figure 5. Immunohistochemical evaluation of IgM-bearing lymphocytes in the lymph nodes of goats. Animals were subjected to sodium arsenite treatment (5 mg/kg orally daily for 15 weeks). A(a): immunohistochemical analysis photographs, T_1, untreated control, and T_2, sodium arsenite-treated. Arrow indicates IgG-positive lymphocytes (600× for both panels). A(b): Graphical representation of percentage (mean + SEM) of IgG-bearing lymphocytes in each group (n = 3 for each group). B: After 15 weeks of exposure, arsenic was stopped and the animals were supplemented with spirulina for another 15 weeks. B(a): T_{3-i}, control (+) (without spirulina); T_{3-ii}, T_{3-iii}, and T_{3-iv} were treated with 50 mg, 100 mg, and 150 mg spirulina/kg body weight, respectively. Arrow indicates IgG positive lymphocytes (600× for all panels). B(b): Graphical representation of percentage (mean + SEM) of IgG-bearing lymphocytes in each group (n = 3 for each group).

within spirulina-supplemented groups. The cutaneous manifestations of chronic arsenic poisoning have an insidious onset. Predominant skin lesions in humans are hyperkeratosis, parakeratosis, acanthosis, and papillomatosis (16). Hyperkeratosis and thickening of the stratum corneum in the skin of arsenic-treated goats is in line with that seen in humans (17). This finding supports the hypothesis that arsenic exposure causes chronic stimulation of keratinocyte-derived growth factors that act to facilitate skin cancer by serving as co-promoters. Destruction of hair follicles in arsenic-treated goats demonstrates that hair follicles are the predilection site of arsenic. However, milder skin lesions than in humans may be due to unexposed areas and short durations of treatment.

Sodium arsenite treatment to the goats did not significantly alter the IgG-bearing lymphocytes in the

lymph node as compared to the control. Supplementation of spirulina to arsenic-treated goats did not significantly modify the IgG-positive to IgG-negative lymphocyte ratio or the IgM-positive lymphocytes in the lymph node. Arsenic has been recognized as a powerful immunomodulatory agent in many laboratory animals and in epidemiological studies (18). However, the insignificant effect of spirulina on immune functions in the present study might be due, in part, to large intake of green grass, which might provide more micronutrients and antioxidants than the small amount of spirulina.

The present study determined that low, subchronic treatment with sodium arsenite in goats resulted in the accumulation of arsenic in tissues, which moderately altered the architecture of tissues but did not affect the IgG- and IgM-bearing lymphocytes. Spirulina showed very little effect on the repair of arsenic-induced lesions

in goats. Rapid restoration of body functions in arsenic-induced goats may be due, at least in part, to the rapid detoxifying capacity of arsenic in goats and/or the antioxidant role of green grass. Additional laboratory studies with chronic higher doses of arsenic and the role of green grass in reducing the effects of arsenic toxicities in goats need to be assessed to substantiate these suggestions.

Over all, spirulina supplementation did not demonstrate any significant therapeutic effects in arsenic-induced toxicities in the Black Bengal goat. Therefore, spirulina may not be effective in treating arsenic toxicosis in small animals such as goats.

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