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ORIGINAL ARTICLE

Aquaculture

Dietary supplementation of *Chlorella vulgaris* ameliorates chronic sodium arsenite toxicity in Nile tilapia *Oreochromis niloticus* as revealed by histopathological, biochemical and immune gene expression analysis

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Abstract

Arsenic toxicity in an aquatic environment is a major concern, and its elimination has become a global challenge. In the current study, histopathology, serum biomarkers and cytokine gene expression were comparatively examined in fish fed with a control diet or diets containing *Chlorella vulgaris* (Ch) after exposure to sodium arsenite ($NaAsO_2$) in Nile tilapia (*Oreochromis niloticus*) with the aim of evaluating the protective role of Ch against arsenite-induced toxicity. Severe histopathological alterations were evident in fish exposed to 7 ppm (parts per million) arsenite for 21 days, compared to unexposed fish. Levels of serum biomarkers ALT, AST, ALP, urea and creatinine were increased, but the levels of Na+, total proteins, albumins and globulins were decreased. Moreover, the expression of all the cytokine genes examined, including IL-1β (7-fold), TNF-α (14-fold) and TGF-β1 (13-fold), were significantly upregulated after arsenite exposure. However, in fish fed with diets containing 5% or 10% Ch, the histopathological alterations in the gills, liver and head kidney were reduced, the biomarkers were stabilized, and the upregulation of cytokine gene expression was lowered, with the high Ch diet (10%) showing more prominent effects. These results suggest the protective and therapeutic roles of Ch as a feed supplement in Nile tilapia against arsenic induced toxicity.

Keywords Fish · Heavy metals · Algae · Immunity · Enzymatic activity · Tissue alterations

Introduction

The contamination of aquatic ecosystems is one of the major threats worldwide affecting aquatic lives. Heavy metal pollution is one of these threats (Ahmed et al. 2013), where the exposure of heavy metals in living organisms leads to various deleterious health hazards such as immunotoxicity, genotoxicity, and nephrotoxicity (Cobbina et al. 2015). Arsenic (As) is one of the most hazardous heavy metals that is released in the aquatic environment through both geogenic and anthropogenic processes (ATSDR 2002). In aquatic habitats, As is biologically available to organisms, including fish, by the aid of several species of microorganisms (Duker et al. 2005; Gonzalez et al. 2006). Fish, in particular, are very sensitive to these pollutants in their environment, and are considered a good model for monitoring potential risks associated with them (Lakra and Nagpure 2009). Arsenite (As^{3+}) and arsenate (As^{5+}) are the predominant types present

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in the aquatic environment and are interconvertible through redox and methylation reactions. In fish, arsenic speciates into two oxidation states, methylated species, arseno-sugars and arsenolipids, that differ in toxicity, and their combinations in fish tissues result in several pathophysiological effects (Kavitha et al. 2010).

Fish are readily susceptible to As toxicity through continuous gill exposure and intake of As-contaminated food (Aruljothi 2014), resulting in accumulation in various tissues. Liver, as the organ responsible for xenobiotic detoxification, is negatively affected by heavy metal contaminated water, which reduces the integrity of hepatocytes and hampers metabolic processes and xenobiotic removal, with subsequent induction of oxidative stress (Guerriero et al. 2010). Reports suggest that chronic As exposure causes abnormal liver function, hepatomegaly, liver fibrosis and cirrhosis in different fish species (Mazumder 2005). Biochemical changes in tissues/organs after As exposure have been reported in freshwater fish fingerlings of *Labeo rohita* (Palaniappan and Vijayasundaram 2009), and in mammals as well (Tripathi and Kumar 2011).

Recently, studies have been focused on the innate immune gene disorders observed in organisms after xenobiotics exposure. In this regard, As exposure has been associated with immunosuppression with increased risk to pathogen invasion (Cobbina et al. 2015). Zebrafish exposed to Cd and Cr resulted in significant upregulation of TNF-α, IL-6 and IL-1β gene expression (Jin et al. 2015). Similar upregulation of gene expression was observed in NADPH oxidase and GPx in macrophages after 24-h exposure to Cu (Teles et al. 2011). Many previous studies have reported on the harmful impacts of low concentration heavy metals on development-related genes of aquatic organisms (McCollum et al. 2014; Soetaert et al. 2007; Thévenod 2009; Wang et al. 2012).

Histopathological analysis is considered to be a good tool for detecting direct effects of xenobiotics within target organs of fish in laboratory experiments (Capkin et al. 2009), that could induce a number of lesions/changes in different organs (Ahmed et al. 2013). Liver and gills are suitable organs for histological investigations in order to assess the effect of pollution (Figueiredo-Fernandes et al. 2007). Pathological alterations have been observed previously in the gills and liver tissues in *Oreochromis mossambicus* exposed to different concentrations of $NaAsO_2$; lesions observed were in the form of epithelial hyperplasia, and there was epithelial lifting and edema, lamellar fusion, aneurism, desquamation and necrosis in gill tissue. The liver tissue showed focal lymphocytic and macrophage infiltration, congestion, vacuolization and shrinkage of hepatocytes, dilation of sinusoids, cloudy swelling, vacuolar degeneration, focal necrosis and nuclear hypertrophy (Ahmed et al. 2013).

The presence of As contaminating water in an aquatic environment is particularly serious in fish, affecting their survival and growth, and even resulting in extinction of entire fish populations in polluted reservoirs, in addition to the human health risks arising from the consumption of contaminated fish with high As content in their tissues (Avigliano et al. 2015). Thus, it is important to develop methods to counteract the toxic effects of heavy metals. In this regard, many physical and chemical methods have been considered, but all were inefficient (Suzuki et al. 2013; Torres-Perez et al. 2012). Microalgae are renowned for their potential role in a variety of applications as live feeds for different growth stages of many aquatic species such as bivalve mollusks, abalone, crustaceans, and some fish species. This is due to the great effects it has on body weight gain, increased triglyceride and protein deposition in muscle, enhanced disease resistance, lowering nitrogen output released into the environment, increased digestibility, physiological activity, starvation tolerance, and carcass quality (Kamat et al. 2000; Sirakov et al. 2015). *Chlorella vulgaris* (Ch), a freshwater microalgae, is rich with many bioactive components such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, and sterols (Soontornchaiboon et al. 2012). Such bioactive components contribute to immunity-modulating, anti-cancer, and protective properties against hematopoiesis, hypertension, cataracts, and age-related diseases. Thus, Ch as GRAS (generally recognized as safe) is used in many medical treatments (Kumar et al. 2018).

The higher content of carotenoids in microalgae has shown not only antioxidative properties but also anti-inflammatory (Soontornchaiboon et al. 2012). Deleterious effects, such as immune suppression, oxidative stress and carcinogenicity, have been observed due to As^{3+} intoxication (Elia et al. 2018). Our previous work also revealed that As^{3+} intoxication suppressed innate immune responses and induced oxidative stress, and found that Ch supplementation had a protective role against As^{3+} intoxication (Zahran and Risha 2014). Therefore, the current study was undertaken to better understand the mechanisms underlying the As^{3+} intoxication through evaluating the histopathological alterations in tissues, cytokines expression analysis, and biochemical parameters, to gain more knowledge regarding the potential protective and therapeutic roles of *Chlorella* dietary supplementation to ameliorate As-induced toxicity in Nile tilapia *Oreochromis niloticus*.

Materials and methods

Chemicals

Sodium arsenite ($NaAsO_2$) with 98% purity was obtained from MERCK (0082970, Art. 6287) and used without further purification for the experiment. A dried powder of

Chlorella vulgaris (Ch) culture was purchased from the Institute of National Research Center, Cairo, Egypt.

Fish maintenance

One hundred and twenty Nile tilapia, (average body weight 90 g), were procured from a private fish farm in Ad-Dakahliya province, Egypt. They were acclimatized for 3 weeks in large tanks provided with adequate aeration and an underwater internal powered filter under laboratory conditions before they were exposed to As treatment. During that period, fish were fed ad libitum with a control basal diet (Table 1), at 25 ± 2 °C, and about 50% of the water was exchanged daily to maintain water quality. No clinical signs were ever observed during this period.

As exposure

The fish were exposed to a sub-lethal concentration of 7 ppm (parts per million) sodium arsenite for up to 21 days. This concentration equates to 1/10th of the median lethal concentration of 71.7 ppm based on a 96-h toxicological assay in Nile tilapia (Hwang and Tsai 1993). A fresh daily stock solution of $NaAsO_2$ was prepared by dissolving the analytical grade $NaAsO_2$ in double-distilled water, and then the desired concentration was prepared from the stock solution. Unexposed fish were maintained in separate tanks without As under identical conditions.

Table 1 The components of the diets (air dry basis, %)

Ingredient	Control diet (Ch0)	Ch diet 1 (Ch5)	Ch diet 2 (Ch10)
Yellow corn	17.65	17.65	17.65
Soybean meal	20.50	20.50	20.50
Fish meal	25.00	25.00	25.00
Wheat bran	34.00	29.00	24.00
Corn gluten meal	1.00	1.00	1.00
Sunflower oil	0.85	0.85	0.85
Vitamins and mineral premix[a]	0.50	0.50	0.50
Salt	0.50	0.50	0.50
Ch powder	0.00	5.00	10.00

[a]Trace minerals and vitamins premixes were prepared to cover the levels of the micro minerals and vitamins for tilapia fish as recommended by NRC, 1993.Vitamins premix (IU or mg kg^{-1} diet); vit. A 5000, vit. D3 1000, vit. E 20, vit. K3 2, vit. B1 2, vit. B2 5, vit. B6 1.5, vit. B12 0.02, pantothenic acid 10, folic acid 1, biotin 0.15, niacin 30. Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 and Se 0.2. Ch: *Chlorella vulgaris*

Preparation of experimental diets

A dried Ch powder was purchased from the Institute of National Research Center, Cairo, Egypt. The components of diets with supplementation of Ch powder are detailed in Table 1. All ingredients were mixed with oil and then with water, resulting in a stiff dough. Each diet was then extruded through a mincer. The resulting strands were shadow-dried, broken up, sieved into pellets, and stored in plastic bags at 4 °C until use.

Experimental design

Nile tilapia were distributed in the aquaria and triplicate tanks were assigned per dietary treatment group (total $N = 120$ fish). Four groups were assigned: a control group fed with the control diet without sodium arsenite in the water (Ch0), and groups with sodium arsenite (7 ppm) in the water that were fed with the control diet (Ch0+As), or diets supplemented with 5% (Ch5+As) or 10% (Ch10+As) Ch powder (Table 1). Fish were fed ad libitum twice daily in equal rations at 09.00 h and 16.00 h for 21 days. Water was changed daily at about 80% during the experimental period and the water quality (temperature 25 ± 2 °C, dissolved oxygen (mg/l) 6.62 ± 0.10, and pH 7.25 ± 0.04) was maintained during the entire experiment. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in the water.

Sample collection

Three fish from each aquarium (9 fish /group) were sampled on days 7, 14 and 21. Each aquarium was sampled one at a time; the fish were sedated with a low dose of buffered tricaine, MS-222 (tricaine methanesulfonate, FINQUEL®, ARGENT) (30 mg/l tricaine + 60 mg/l sodium bicarbonate), and each fish was then euthanized one at a time in a separate container.

For determination of serum biochemical parameters, fish were bled from the caudal peduncle. Blood was transferred immediately to a plain centrifuge tube and centrifuged at 1700×*g* for 15 min at 4 °C. The serum was separated carefully and frozen at − 80 °C until use. For histopathology, the gills, liver and head kidney tissues were excised and fixed immediately in 10% neutral buffered formalin. For gene expression analysis, the liver tissue was collected at the end of the exposure period (21 days) and immediately placed in RNAlater (Sigma) and stored at − 20°C, until further use.

Semi-quantitative histopathological analysis

The gills, liver and head kidney were dehydrated in a graded series of ethanols after formalin fixation, cleared in xylene

and embedded in paraffin. Sections (5 µm) were cut and stained with hematoxylin and eosin for the histopathological examination (Bancroft et al. 1996). Lesions were evaluated semi-quantitatively by ranking tissue lesion severity. Ranking from 0 to 3 depended on the degree of tissue alteration (DTA) as follows: (0) absent: structure of the tissue is normal with no lesion present, (1) lesions present in < 20% of each studied field, (2) lesions present in between 20 and 60% of each studied field, and (3) lesions present in > 60% of each studied field. This ranking was used according to Benli et al. (2008) to establish an overall assessment value of the histopathological lesions for each individual fish tissue.

Serum biochemical parameters

The activities of serum aspartate aminotranseferase (AST), alanine aminoranferase (ALT), and alkaline phosphatase (ALP) were estimated using commercial kits (Randox, UK). The concentration of serum total proteins, albumins and globulins were measured spectrophotometrically using test kits (Stanbio, USA). The serum sodium (Na^+) and potassium (K^+) levels were measured spectrophotometrically using test kits (Teco–Diagnostics, USA). The levels of serum urea and creatinine were measured using available kits (Diamond, Egypt and Human, Germany).

Extraction of total RNA

Total RNA was extracted from liver samples using an RNeasy kit according to manufacturer's instructions (QIAGEN, Germany). Briefly, 100 mg of tissues was homogenized in 600 µl of the lysis buffer RLT (2-mercaptoethanol was added to buffer RLT before use) in a mortar. The lysate was centrifuged at 14,000 rpm for 2 min. Absolute ethanol (400 µl) was added to the cleared supernatant and mixed well by pipetting. The sample was transferred to an RNeasy spin column placed in a 2-ml collection tube and centrifuged for 1 min at 10,000 rpm. The filtrate was discarded and the RNeasy spin column was washed by adding 700 µl buffer RW_1, then centrifuged for 1 min at 10,000 rpm. The washing step was repeated twice with 500 µl buffer RPE and centrifuged for 1 min at 10,000 rpm. The RNeasy spin column was transferred to a new tube and centrifuged for 1 min at full speed. The RNA was eluted by adding 40 µl RNase-free water directly to the spin column membrane. The column was incubated at room temperature for 1 min, then centrifuged at 8000 rpm for 1 min, and the RNA was stored at − 80 °C.

cDNA synthesis and real-time PCR analysis of gene expression

The total RNA was converted to cDNA using RevertAid™ Reverse Transcriptase (Fermentas), following the manufacturer's instructions. Q-PCR was performed using SYBR Green PCR Master Mix (Fermentas, USA). Each reaction was performed in a 25-µl mixture, which contained 1 µl of 10 pmol/µl of each primer, 1 µl of template cDNA (conc. 50 ng), 12.5 µl of 2X SYBR Green PCR Master Mix and 9.5 µl of nuclease-free water. Samples were spun before being loaded in the rotor wells, and each sample was run in triplicate. The amplification program proceeded at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s; annealing at 60 °C for 30 s and extension at 72 °C for 30 s, using a Rotor-Gene 6000 cycler (QIAGEN, ABI System, USA). After the cycling protocol, the melting curves were obtained to assess the specificity. The expression of pro-inflammatory cytokines IL-1β, TNF-α and anti-inflammatory cytokine TGF-β1, as well as the housekeeping gene elongation factor-1α (EF-1α), were examined. The primers for real-time PCR are detailed in Table 2, with at least one primer of a pair designed to cross an intron so that genomic DNA could not be amplified under the PCR conditions used. The expression of each gene was first normalized to that of EF-1α, and presented as a fold change by calculating the average expression level of the arsenite exposed samples divided by that of the controls as described previously (Wangkahart et al. 2016).

Ethical statement

This study was conducted in accordance with strict guidelines for the use of laboratory animals. All experimental procedures were in compliance with the Animal Care and Use Guidelines at Mansoura University and approved by the local Administrative Panel on the Laboratory Animal Care Committee. All treatments were performed under tricaine

Table 2 Primers used for real-time PCR analysis of gene expression

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)	Accession number
IL-1β	CTGTGTGACAACTCTCAGAAAGATCTTG	TGTCATCATGGTATTGCTCCAGAA	XM_003452995
TNF-α	GAATACAAGGCCAGAAAGGATGACAC	GACCTTTTGAGTCGCTGCCTTC	NM_001279533
TGF-β1	CAGGAAAGATCTAGGATGGAAGTGGA	TGGACAGCTGCTCGACCTTGTG	XM_003459454
EF-1α	GGTGTTGGAGAGTTCGAGGCTG	TCCCTTGAACCAGCTCATCTTGTC	HE608771

anesthesia and were performed so as to minimize suffering and stress.

Statistical analysis

Data of serum biochemical parameters were subjected to one-way analysis of variance (ANOVA) using the SPSS computer software (SPSS version 17.0 for Windows). Serum biochemical parameters and gene expression levels were graphically presented using GraphPad Prism version 6 (GraphPad Software Inc.). Differences between means were assessed by Duncan's multiple-range test and effects with a probability of $P < 0.05$ were considered significant. Scores of histopathological variables were tested by the chi-square test among all groups regardless of time.

Results

Ch dietary supplementation ameliorated histopathological alterations after arsenite exposure

The main histopathological changes in gills of fish exposed to 7 ppm sodium arsenite included epithelial hyperplasia, fusion of secondary lamellae, lifting up of the epithelium, and aggregations of inflammatory cells in gill filaments. Additional changes were inconsistently seen, such as edema, congestion, and focal hemorrhage at primary lamellae (Fig. 1a–d). Frequencies of histopathological lesions observed in gills were significant between all groups with no significance over time, (chi-square (χ^2) test: $\chi^2 = 99.9$, 195.6, 163, and 94.9, respectively, df = 9, $P < 0.0001$). However, these frequencies of lesions were reduced greatly in the Ch10+As group (Table 3).

Liver of the exposed group had hepatocyte hypertrophy, cytoplasmic vacuolation, nuclear pyknosis and karyorrhexis, focal lymphocytic and macrophage infiltration, peliosis hepatitis, and congestion and dilation of sinusoids. The pancreatic cells demonstrated vacuolization, decreased basophilia, shrinkage, and disorganization. Necrosis was evident in both hepatocytes and hepatopancreas (Figs. 2, 3 and 4). By day 21, the sinusoids almost regained their normal structure, and the compactness of the pancreatic tissue was found to reappear. The cytoplasm of the biliary epithelium was swollen and vacuolized. Similar histopathological changes were observed in groups Ch5+As and Ch10+As. DTA observed in livers of Nile tilapia fish was significant between all groups with no significance over time (chi-square test: $\chi^2 = 108.3$, 112.5, 82.8, and 104, 157.9, respectively, df = 9, $P < 0.0001$) (Table 4). Similarly to gills, the frequencies of the highest score for the lesions detected in liver were reduced in the Ch10+As group (Table 4).

Head kidneys showed depletion of the hematopoietic precursors, necrosis in the hematopoietic elements, thickening of the basal endothelial membrane, disruption of tissue integrity, edema, and sinusoidal blood congestion upon As exposure. Degeneration and necrosis of melano-macrophage centers (MMC) were rarely seen (Fig. 5a–d). Similar lesions were found in treated groups (Ch5+As and Ch10+As) but to

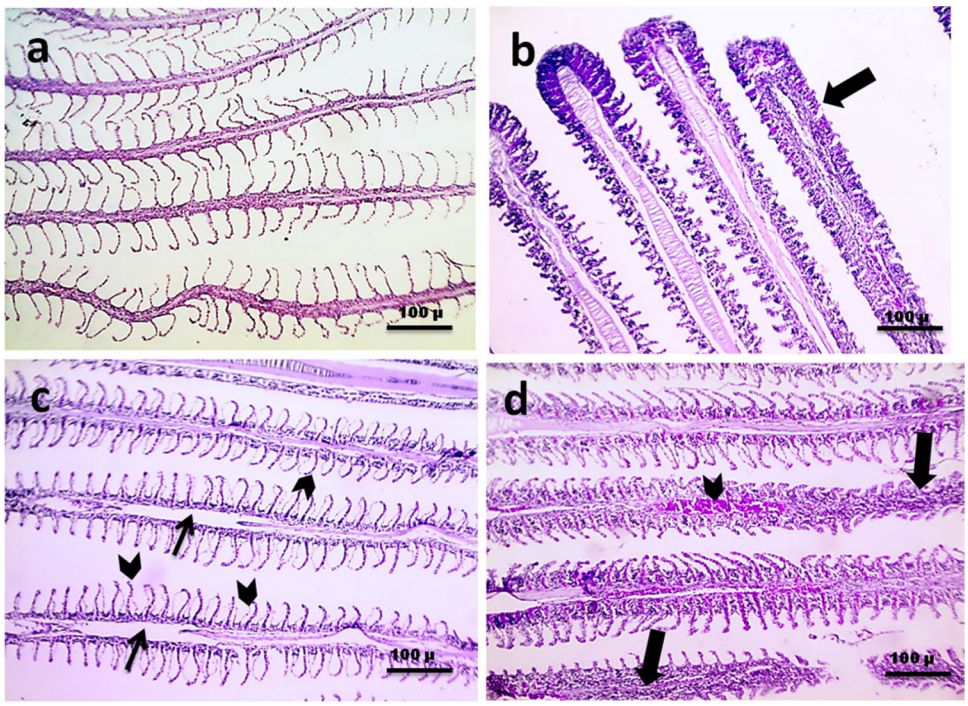

Fig. 1 **a–d** Histopathology of sodium arsenite exposure in the gills of Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0+As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) after 21 days exposure to sodium arsenite. **a** Control gill filaments show normal histology. **b** Gill filaments of fish exposed to arsenic show primary filament hyperplasia and fusion of secondary lamellae (*arrows*). **c** Lifting up of the epithelium (*arrowheads*), and edema (*arrows*). **d** Leukocytic cells infiltration (*arrows*) and congestion (*arrowhead*) (H & E ×100)

Table 3 Lesions ranking by stage based on histological appearance of the gills of the control and experimental Nile tilapia (*Oreochromis niloticus*) after 21 days exposure of sodium arsenite

Treatment	Primary filament hyperplasia		Fusion of secondary lamellae		Sub-epithelial edema		Leukocytic infiltration	
	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)
Ch0	0:26	58	0:45	100	0:45	100	0:44	98
	1:19	42	1:0	0	1:0	0	1:1	2
	2:0	0	2:0	0	2:0	0	2:0	0
	3:0	0	3:0	0	3:0	0	3:0	0
Ch0+As	0:0	0	0:0	0	0:0	0	0:9	20
	1:9	20	1:4	9	1:2	4	1:12	27
	2:21	47	2:29	64	2:22	49	2:15	33
	3:15	33	3:12	27	3:21	47	3:9	20
Ch5+As	0:3	7	0:3	7	0:5	11	0:13	29
	1:16	35	1:11	24	1:12	27	1:19	42
	2:23	51	2:31	69	2:21	46	2:12	27
	3:3	7	3:0	0	3:7	16	3:1	2
Ch10+As	0:8	18	0:4	29	0:7	16	0:18	40
	1:15	33	1:19	19	1:15	33	1:22	49
	2:21	47	2:22	45	2:18	40	2:5	11
	3:1	2	3:0	7	3:5	11	3:0	0
Chi-square value, X^2	99.9		195.6		163		94.9	
Degree of freedom	9		9		9		9	
P value	*< 0.0001*		*< 0.0001*		*< 0.0001*		*< 0.0001*	

[a]Scoring system of histopathological changes in the gills: 0 (absent: structure of the gills is normal. with no lesion present), 1 (lesion is present in less than 20% of studied fields), 2 (lesion is present between 20% and 60% of studied fields) and 3 (lesion is present in more than 60% of studied fields)

a lesser extent. In the same trend, DTA observed in head kidneys was significant between all groups with no significance over time, (chi-square test: $\chi^2 = 87.9$, 134.9, 27.6, respectively, df = 9, $P < 0.0001$). Similarly, the frequencies of the highest score for the lesions detected in head kidneys were reduced in the Ch10+As group (Table 5).

Ch dietary supplementation mitigated the upregulation of enzyme activity after arsenite exposure

Toxicant stress can lead to elevation of enzyme activities which are considered to be indicators of hepatic tissue damage and dysfunction, as previously described by Datta et al. 2007 and Ozmen et al. 2006, and verified in our experiment. There was no change of activities of AST, ALT and ALP in control fish during the time course fed with the control diet Ch0 (Fig. 6). Seven parts per million sodium arsenite exposure in fish fed with the diet Ch0+As significantly increased the plasma ALT and ALP activities at days 7, 14 and 21, and the AST activity at day 14 and 21. Five percent (Ch5+As) and 10% (Ch10+As) Ch supplementation significantly inhibited the arsenite-increased activities of all three enzymes (Fig. 6). The ALT activity in Ch5+As and Ch10+As fed fish wasn't altered at day 7 and was lower than Ch0+As fed fish 14 days post arsenite exposure. Although the Ch5+As fed fish had a higher level of ALT activity at day 21, the Ch10+As fed fish had the same level of ALT activity as the control (Fig. 6a). The AST activity in 5% and 10% fed fish remained unchanged from 7 to 21 days except in 5% Ch fed fish at day 14 when the AST activity was higher than in control (Ch0) but lower than in Ch0+As fed fish (Fig. rb). The ALP activity in 5% and 10% Ch fed fish also remained unchanged at all time points tested (Fig. 6c).

Ch dietary supplementation counteracted the down-regulation of plasma proteins after arsenite exposure

It had been previously demonstrated that toxicant stress negatively affects the plasma protein levels (Samuel et al. 2005). The fish exposed to As (Ch0+As) showed

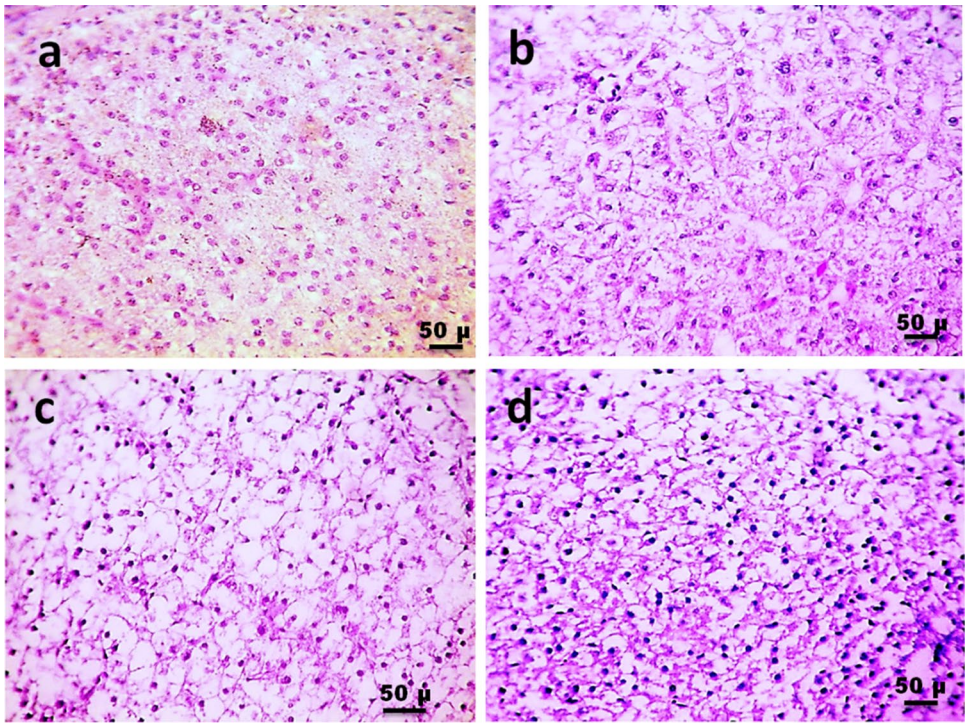

Fig. 2 **a**–**d** Hepatocyte vacuolization in the liver of Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0+As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) after 21 days. **a** Control liver with score 0 shows normal histology. **b** Score 1 shows mild cytoplasmic vacuolization. **c** Score 2 shows moderate cytoplasmic vacuolization. **d** Score 3 shows severe cytoplasmic vacuolization with diffuse nuclear pyknosis (H & E ×200)

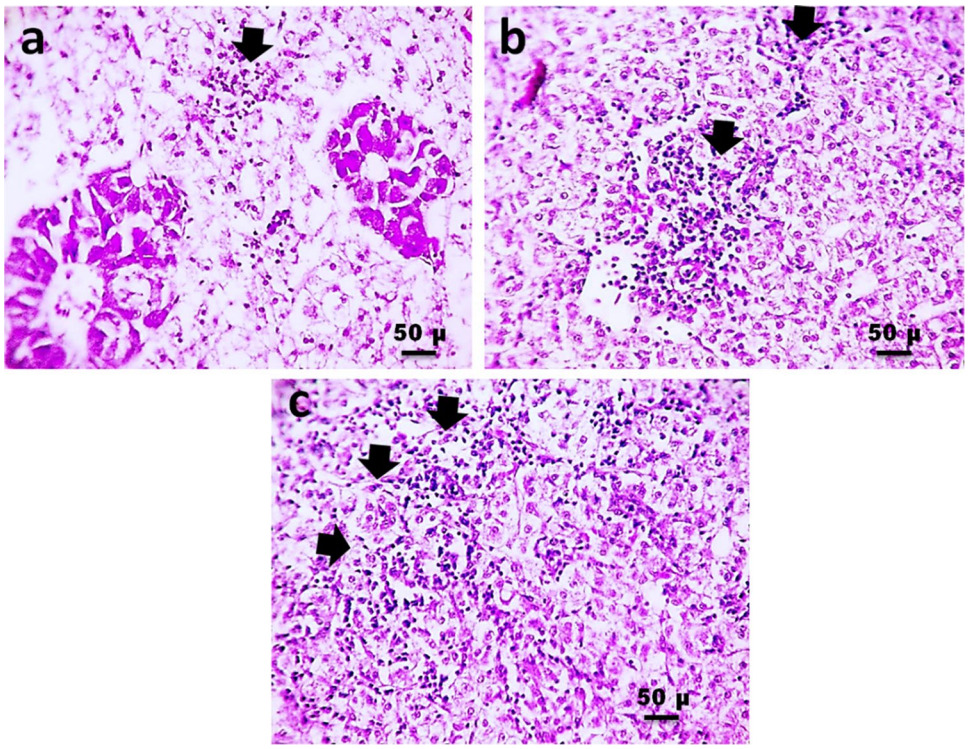

Fig. 3 **a**–**c** Liver inflammation of Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) after 21 days. **a** Score 1 demonstrates mild leukocytic cells infiltration (*arrow*). **b** Score 2 demonstrates moderate leukocytic cells infiltration (*arrows*) **c** Score 3 demonstrates severe diffuse leukocytic cells infiltration (*arrows*) (H & E ×200)

a significant reduction in plasma protein level at days 14 and 21. The albumin level was nominally decreased at days 7 and 14 while it was significantly lowered at day 21 in arsenite exposed fish. The globulin level was nominally decreased at days 7 and 21 but was significantly lowered at day 14 in the arsenite-exposed group. No changes of total protein, albumin and globulin levels were observed in arsenite exposed fish fed with Ch supplemented diets at both doses used (Fig. 7a–c).

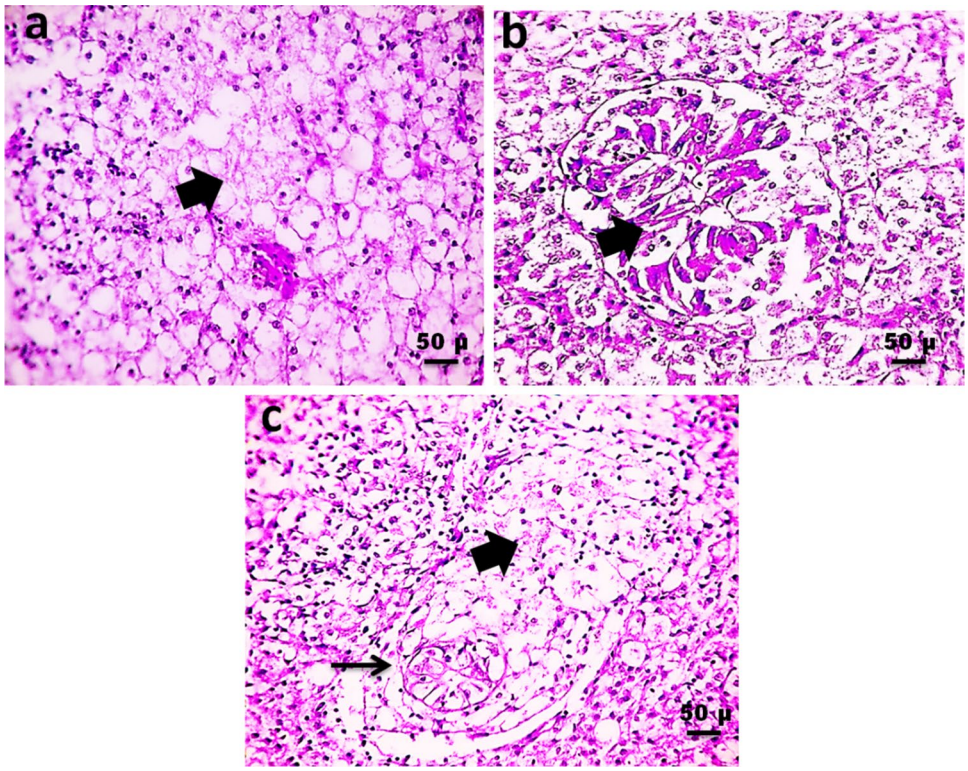

Fig. 4 **a**–**c** Histopathology of sodium arsenite exposure in the liver of Nile tilapia at day 14. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) after 14 days. **a** Focal area of necrosis (*arrow*), **b** Decreased basophilia, shrinkage and necrosis in hepatopancreas (*arrow*), **c** severe vacuolization of pancreatic cells (*short thick arrow*) and biliary epithelium (*long thin arrow*) (H & E ×200)

Ch dietary supplementation counteracted the increased plasma urea and creatinine after arsenite exposure

Kidney biomarkers, such as plasma urea and creatinine, were significantly increased, as previously indicated, in response to other heavy metals that elucidate the toxicant action in impairing kidney function (Elgaml et al. 2015). Arsenite exposure significantly increased the plasma urea concentration from 7 to 21 days and creatinine concentration at 14 and 21 days in fish fed the control diet (Ch0). In contrast, both urea and creatinine concentrations remained unchanged in arsenite exposed fish fed with Ch supplemented diets at both doses used (Fig. 8a, b).

Ch dietary supplementation counteracted the down-regulation of plasma electrolytes after arsenite exposure

Maintaining plasma electrolytes is important for many physiological functions and these electrolytes are very sensitive to environmental stressors and commonly modified upon pollutants exposure (Suvetha et al. 2010). As expected, the level of Na^+ decreased significantly in arsenite exposed fish fed with the control diet (Ch0+As) from 7 to 21 days but remained unchanged when fed with Ch supplemented diets at both doses used (Fig. 9a). However, K^+ levels showed no significant difference between different groups at the same time points (Fig. 9b).

Ch dietary supplementation counteracted the upregulation of cytokine gene expression after arsenite exposure

A heightened expression of pro-inflammatory cytokines, such as IL-1β and TNF-α, and anti-inflammatory cytokine, such as TGF-β1, indicates a disturbance of the immune system. The liver, by receiving blood both via the portal vein containing dietary antigens, toxins as well as pathogens from the gastro-intestinal tract, and via the hepatic artery containing antigens, pathogens and metastasizing cells from systemic blood circulation, represents a major immune organ of vertebrates (Möller et al. 2014). Hence, the arsenite and diet modulated cytokine gene expression was studied in this tissue at 21 days post arsenite exposure. Arsenite exposure significantly upregulated the expression of Nile tilapia IL-1β (7-fold), TNF-α (14-fold)), and TGF-β1 (13-fold) compared to the control diet Ch0 (Fig. 10). The heightened cytokine gene expression was reduced significantly in 10% Ch fed fish, with a lessened IL-1β expression also seen in 5% Ch fed fish.

Table 4 Lesions ranking by stage based on histological appearance of the liver of the control and experimental Nile tilapia (*Oreochromis niloticus*) after 21 days exposure of sodium arsenite

Treatment	Cytoplasmic vacuolation		Focal necrosis		Leukocytic infiltration		Peliosis hepatis		Congestion	
	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/ examined fields	Percentage showing this lesion (%)
Ch0	0:37	82	0:45	100	0:45	100	0:42	93	0:45	100
	1:8	18	1:0	0	1:0	0	1:3	7	1:0	0
	2:0	0	2:0	0	2:0	0	2:0	0	2:0	0
	3:0	0	3:0	0	3:0	0	3:0	0	3:0	0
Ch0+As	0:0	0	0:6	13	0:5	11	0:5	11	0:1	2
	1:6	13	1:25	56	1:27	60	1:28	62	1:13	29
	2:16	36	2:7	16	2:3	7	2:7	16	2:17	38
	3:23	51	3:7	15	3:10	22	3:5	11	3:14	31
Ch5+As	0:6	13	0:6	13	0:15	33	0:9	20	0:3	7
	1:6	14	1:28	62	1:29	65	1:27	60	1:20	44
	2:19	42	2:8	18	2:1	2	2:7	16	2:17	38
	3:14	31	3:3	7	3:0	0	3:2	4	3:5	11
Ch10+As	0:10	22	0:10	22	0:29	64	0:20	44	0:3	7
	1:10	23	1:29	65	1:16	36	1:23	52	1:19	42
	2:19	42	2:6	13	2:0	0	2:2	4	2:17	38
	3:6	13	3:0	0	3:0	0	3:0	0	3:6	13
Chi-square value, X^2	108.3		112.5		82.8		104		157.9	
Degree of freedom	9		9		9		9		*9*	
P value	*< 0.0001*		*< 0.0001*		*< 0.0001*		*< 0.0001*		*< 0.0001*	

[a] Scoring system of histopathological changes in the liver: 0 (absent: structure of the kidney is normal. with no lesion present), 1 (lesion is present in less than 20% of studied fields), 2 (lesion is present between 20% and 60% of studied fields) and 3 (lesion is present in more than 60% of studied fields)

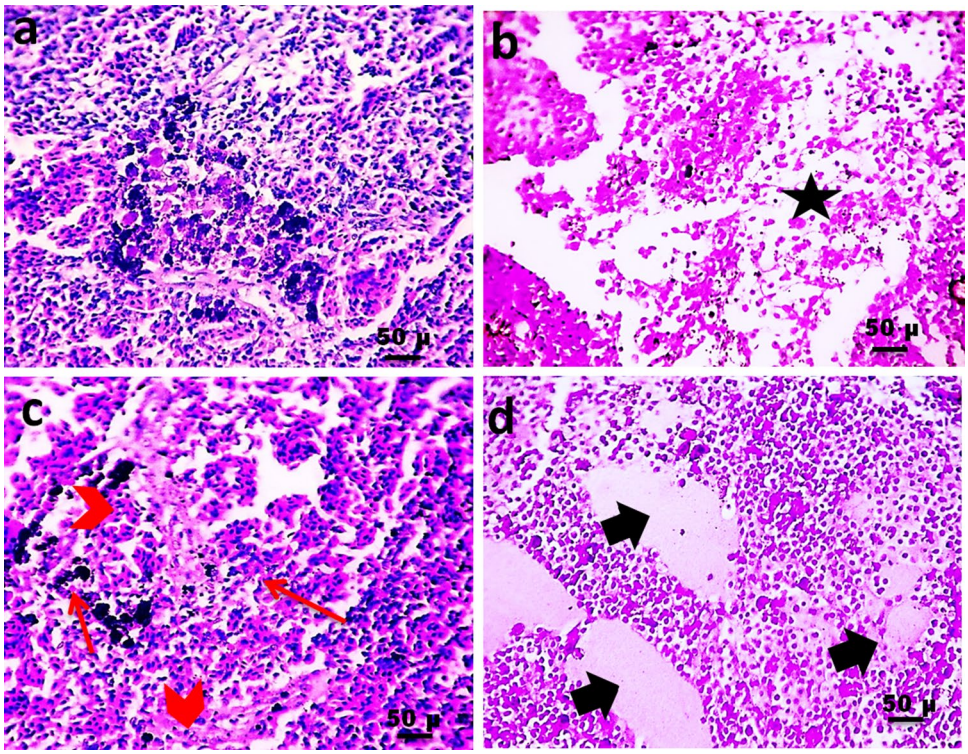

Fig. 5 **a**–**d** Histopathology of sodium arsenite exposure in the head kidney of Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0+As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) after 21 days. **a** Control head kidney shows normal histology. **b** Depletion of the hematopoietic precursors, necrosis in the hematopoietic elements and disruption of tissue integrity (*asterisk*). **c** Thickening of the basal endothelial membrane (*red arrowheads*) and sinusoidal blood congestion (*red arrows*). **d** Edema (*black arrows*) (H & E ×200)

Table 5 Lesions ranking by stage based on histological appearance of the head kidney of the control and experimental Nile tilapia (*Oreochromis niloticus*) after 21 days exposure of sodium arsenite

Treatment	Sinusoidal congestion		Necrosis in the hematopoietic elements		Necrosis of melanomacrophage centers (MMC)	
	Severity of lesion[a]/examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/examined fields	Percentage showing this lesion (%)	Severity of lesion[a]/examined fields	Percentage showing this lesion (%)
Ch0	0:45	100	0:45	100	0:45	100
	1:0	0	1:0	0	1:0	0
	2:0	0	2:0	0	2:0	0
	3:0	0	3:0	0	3:0	0
Ch0+As	0:12	27	0:0	0	0:34	76
	1:3	7	1:3	7	1:0	0
	2:15	33	2:19	42	2:7	15
	3:15	33	3:23	51	3:4	9
Ch5+As	0:9	20	0:6	13	0:39	87
	1:4	9	1:7	16	1:3	7
	2:23	51	2:20	44	2:2	4
	3:9	20	3:12	27	3:1	2
Ch10+As	0:15	33	0:11	24	0:42	93
	1:4	9	1:10	22	1:2	5
	2:23	51	2:16	36	2:0	0
	3:3	7	3:8	18	3:1	2
Chi-square value, X^2	87.9		134.9		27.6	
Degree of freedom	9		9		9	
P value	*< 0.0001*		*< 0.0001*		*< 0.001*	

[a] Scoring system of histopathological changes in the head kidney: 0 (absent: structure of the head kidney is normal, with no lesion present), 1 (lesion is present in less than 20% of studied fields), 2 (lesion is present between 20% and 60% of studied fields) and 3 (lesion is present in more than 60% of studied fields)

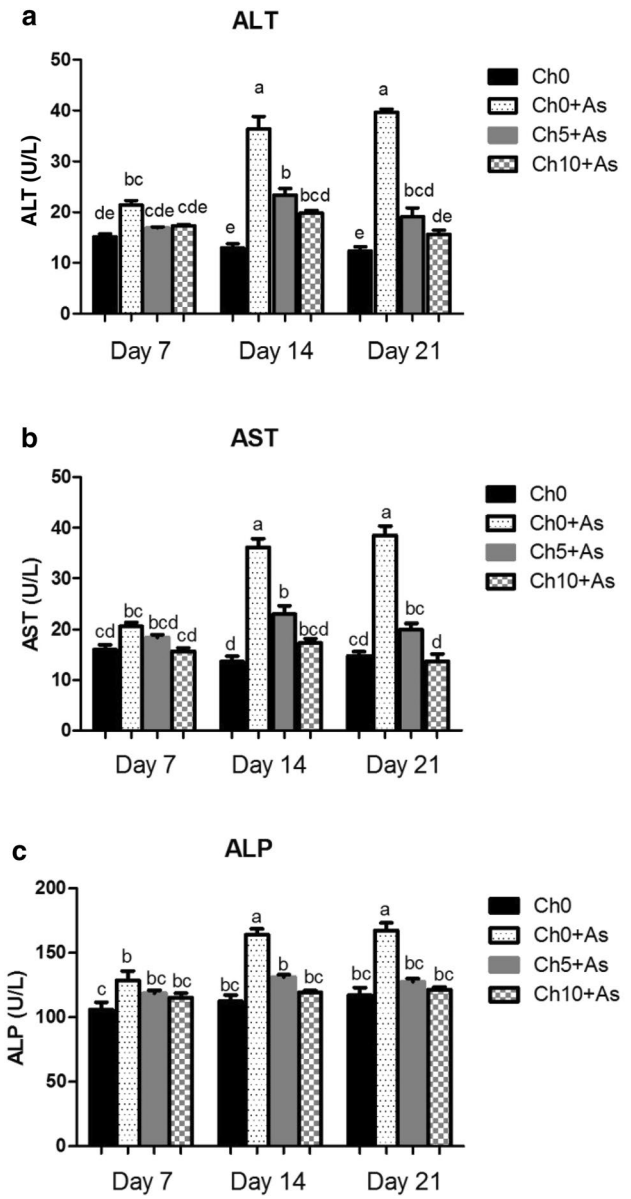

Fig. 6 **a–c** The effects of sodium arsenite exposure and diets on ALT, AST and ALP levels in Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) for 7, 14 and 21 days. The levels of plasma ALT, AST and ALP activities were measured. Data is expressed as the mean ± SEM of six fish. The levels between different groups are statistically significant ($P < 0.05$) where *letters* over the bars are different, as determined by one-way ANOVA

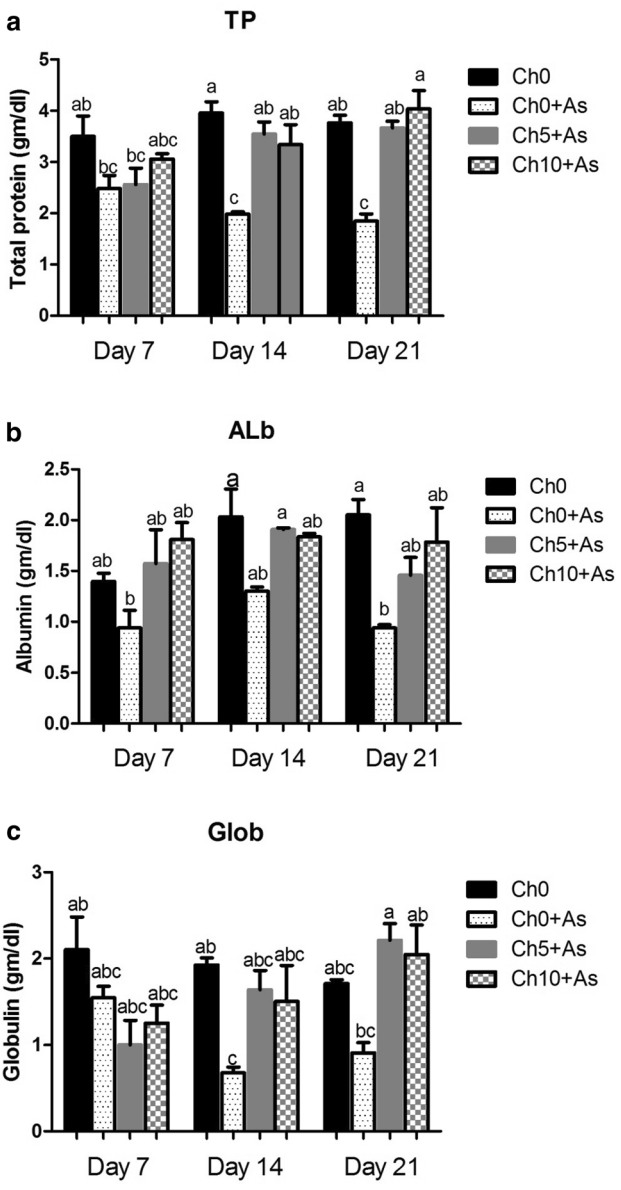

Fig. 7 **a–c** The effects of sodium arsenite exposure and diets on serum total protein levels in Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) for 7, 14 and 21 days. The levels of serum total protein, albumin and globulin were measured. Data is expressed as the mean ± SEM of six fish. The levels between different groups are statistically significant ($P < 0.05$) where *letters* over the bars are different, as determined by one-way ANOVA

Discussion

Great concern has been focused on heavy metals and other pollutants that can be harmful for ecosystems. Fish are very sensitive to such pollutants, leading to various deleterious effects upon entering their organs, due to unremitting exposure (Aruljothi 2014). Thus, fish are considered to be biological indicators for monitoring heavy metal contamination (Jancsó and Hermesz 2015). Heavy metal pollution warrants a new strategy to alleviate such adverse effects. In this respect, removal of toxic metals through biosorption, based on metal binding capabilities of algae and other aquatic plants have been adopted (Kamat et al. 2000).

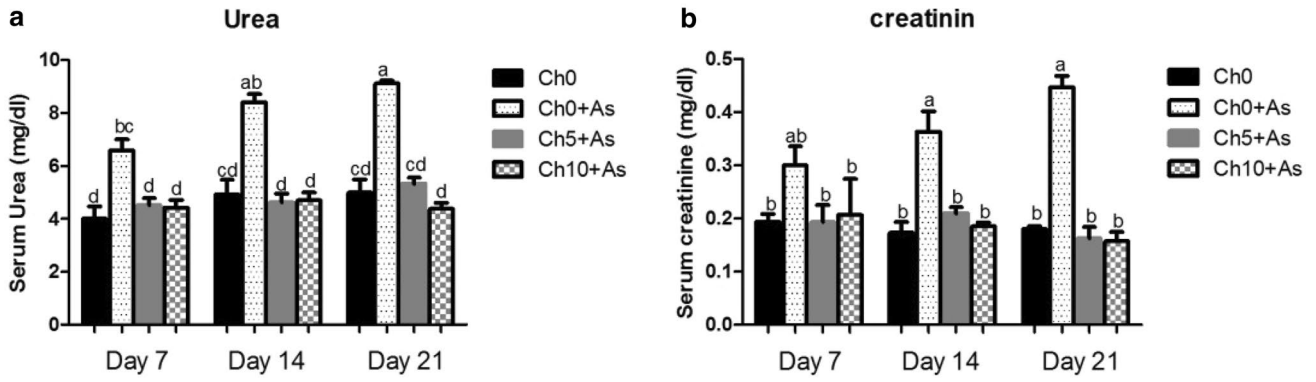

Fig. 8 **a**–**b** The effects of sodium arsenite exposure and diets on serum urea and creatinine levels in Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) for 7, 14 and 21 days. The serum urea and creatinine levels were measured. Data is expressed as the mean ± SEM of six fish. The levels between different groups are statistically significant ($P < 0.05$) where *letters* over the bars are different, as determined by one-way ANOVA

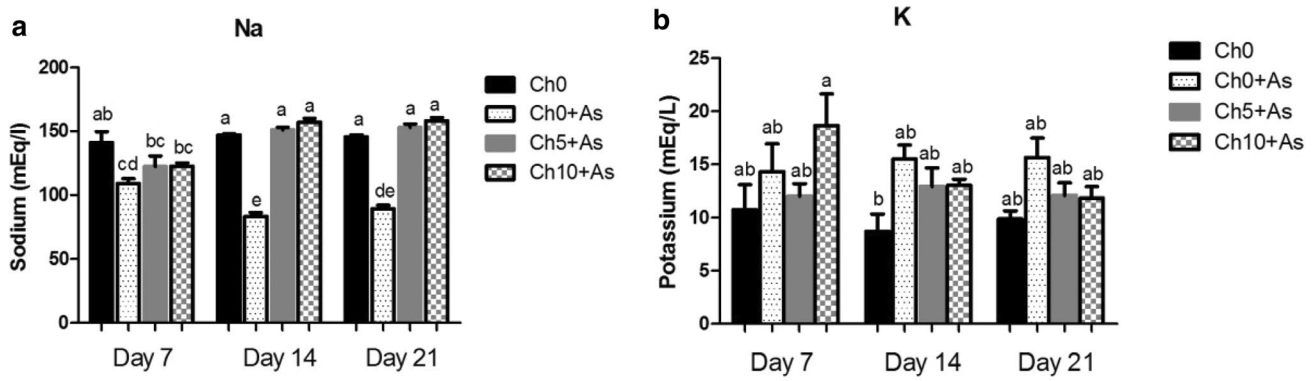

Fig. 9 **a**–**b** The effects of sodium arsenite exposure and diets on serum electrolytes levels in Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) for 7, 14 and 21 days. The levels of serum sodium and potassium were measured. Data is expressed as the mean ± SEM of six fish. The levels between different groups are statistically significant ($P < 0.05$) where *letters* over the bars are different, as determined by one-way ANOVA

In the current study, it was observed that As^{3+} exposure has deleterious effects on serum biochemical parameters of tilapia fish. The results clearly indicated that As^{3+} exposure significantly increased ALT, AST, and ALP levels and decreased total protein levels, indicating damage in liver tissue by As^{3+}. However, in groups that were fed with Ch supplemented diets, these changes in ALT, AST, APL and protein levels were inhibited ($P < 0.05$), with the higher Ch diet showing more prominent effects on maintaining these biomarkers. Our findings of arsenite effects on liver biomarkers were in line with Lavanya et al. (2011) who found a significant increase in GOT (glutamic oxaloacetic transaminase) and decrease in plasma total protein after arsenic trioxide exposure. Additionally, Vetrivel et al. (2014) reported a decrease in total protein level and ALP activity in *Clarias batrachus* after arsenic trioxide exposure. Moreover, Fırat et al. (2011) reported significant increases in ALT, AST and ALP activities in response to Cu, and Pb after 21 days. Elgaml et al. (2015) reported a significant decrease in serum total protein in Nile tilapia exposed to lead acetate at a dose of 73.40 mg/l for 10 weeks. Lowered levels of the protein content in liver and muscle of Nile tilapia were also recorded upon exposure to aluminum sulphate (Al^{3+}) (Correia et al. 2010). Previous studies (Harvey et al. 1994; Karan et al. 1998; Vaglio and Landriscina 1999) were also supportive of our results. These results could be attributed to the toxic effect of heavy metals on liver, which led to degenerative changes, and hypofunction of the liver. Liver damage caused cellular enzymes to be released from the cells or

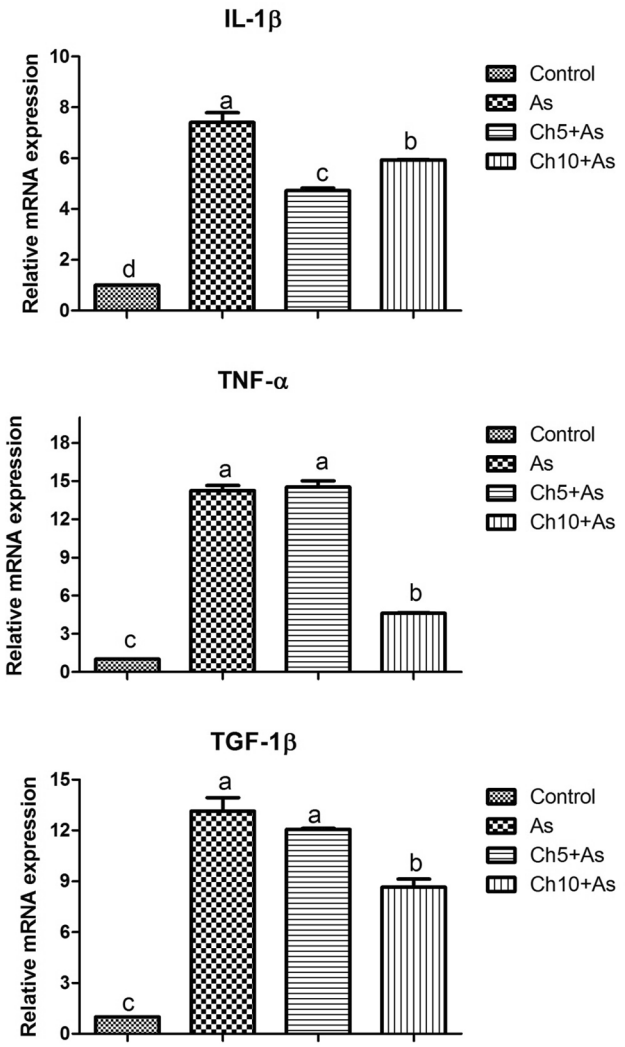

Fig. 10 The effects of sodium arsenite exposure and diets on gene expression of proinflammatory cytokines (IL-1β, TNF-α, and TGF-β1) determined by real-time PCR in liver of Nile tilapia. The fish were fed with the control diet (Ch0) or exposed to 7 ppm sodium arsenite and fed with the control diet (Ch0 + As), or with diets containing 5% *Chlorella* (Ch5+As) or 10% *Chlorella* (Ch10+As) for 21 days. Data is expressed as the mean ± SEM of six fish. The levels between different groups are statistically significant ($P < 0.05$) where *letters* over the bars are different, as determined by one-way ANOVA

leakage of these enzymes from the liver into the blood (Fırat et al. 2011). ALP is involved in transphosphorylation reactions and mediates membrane transport; therefore, a higher level indicates dephosphorylation of biomolecules with a hazardous effect on the energy budget of the cell and subsequent ionic imbalance (Sharma et al. 2012). Hypoproteinemia reported in this study might arise either from kidney dysfunction with protein excretion in the urine, or due to inhibition of the hepatic protein synthesis that is responsible for reducing the synthesis of total proteins (Aly et al. 2015). The homeostatic effects of Ch supplemented diets on the liver biomarkers against the toxic effects of arsenite exposure might be due to the maintained level of membrane-bound enzymes and the activities of antioxidant enzymes near normal levels, confirming their effects as antioxidants and preventing enzyme leakage into the circulation (Elgaml et al. 2015). Besides, Ch is rich in carotenes and other bioactive components such as violaxanthin that exhibit strong antioxidant properties in oxidative stress and function as a chain breaking antioxidant in lipid peroxidation (Sharma et al. 2012; Soontornchaiboon et al. 2012).

Higher levels of kidney markers, urea and creatinine, were evident in the Ch0+As group compared to other groups, and are indicative of kidney dysfunction as a result of glomerular insufficiency (Abdel-Tawwab et al. 2007). Also, there was an increase in the production of reactive oxygen species (Elia et al. 2018; Zahran and Risha 2014) and kidney injury induced by the toxicant (Upasani and Balaraman 2003). Feed supplemented groups Ch5+As and Ch10+As exhibited an improvement in kidney function markers. This might be due to the breakdown of free radicals with subsequent formation of relatively unreactive radical species to protect the kidney tissue from peroxidative damage (Özkan-Yılmaz et al. 2014).

Ionic imbalance was observed in the present study, where Na^+ levels were decreased ($P < 0.05$) in the Ch0+As group compared to other groups. Similar findings were reported by Özkan-Yılmaz et al. (2014) in *Cyprinus carpio* exposed to low pH 4.0 with low (normal water) calcium 6 mg/l and low pH 4.0 with added calcium 15 mg/l treatment for a period of 96 h. Kabilan et al. (2013) corroborated the same findings in *Lates calcarifer* exposed to $HgCl_2$ and $CdCl_2$ at concentrations of 3.5 ppm and 4.0 ppm and their combination at 3 ppm for 35 days. The same trend was reported by Fırat et al. (2011) in Nile tilapia after Cu 0.05 mg/l and Pb 0.05 mg/l exposure for 21 days. This could result from different toxicant actions on organs involved in osmoregulation, metabolism, active transport processes, or the endocrine system (Martinez and Cólus 2002). Pathological alterations can be induced by the toxicant in organs responsible for the exchange of ions between the fish and the surrounding water, or could be attributed to the reduction of Na^+/K^+-ATPase activity, which has a key role in whole body ion regulation (Fırat et al. 2011). Additionally, kidney dysfunction further contributed to ionoregulatory failure through different mechanisms, such as defects in ion reabsorption, proximal tubular cells and tubular dysfunction with hypocalcemia and hypophosphatemia (Patel et al. 2006). Additionally, disturbance in Ca^{2+} homeostasis may lead to disruption of the mechanisms of ion regulation (Moe 2008). Once again, the protective effects of a Ch supplemented diet were observed in regulation of the ionic balance owing to its biologically active components, especially carotenoids that revealed strong antioxidant properties in oxidative stress and function

as a chain breaking antioxidant in lipid peroxidation, maintaining ionic regulation (Sharma et al. 2012; Soontornchaiboon et al. 2012; Özkan-Yılmaz et al. 2014).

Cytokines have important roles in the regulation of the fish immune response. Our results revealed upregulation of all analyzed cytokines, IL-1β, TNF-α, and TGF-β1 in the Ch0+As exposed group compared to the control group (Ch0). This is consistent with the findings of Teles et al. (2011), where upregulation of expression of IL-1β, TNF-α and IL-6 mRNA was seen in rainbow trout head kidney macrophages exposed to copper. Similar results were seen in increased expression of other immune-related genes (e.g., TGF-β), in the whole head kidney of *Morone saxatilis* (Geist et al. 2007) and *Solea senegalensis* (Prieto-Álamo et al. 2009) upon copper exposure in vivo. Upregulation of IL-1β expression in the present study was due to the system's response to As^{3+} exposure. Similar IL-1β upregulation was noted against a low level of As (2 or 10 ppb) exposure (Nayak et al. 2007), and in acute promyelocytic leukemia cells (Jiang et al. 2003). In this study, TNF-α gene expression was significantly upregulated upon As^{3+} exposure as well. TNF-α is involved in many systemic inflammations and its upregulation is a response reflection of As^{3+} exposure. This result was similar to what had been found by Yu et al. (2002), who recorded TNF-α induction from mononuclear cells exposed to a low arsenic concentration, and 1 µM As led to a cytotoxic effect on T-cells. General upregulation of TNF- α, IL-6, and IL-1β was noted in the kidney of adult rats upon acrylamide (ACR) and aluminum (Al^{3+}) exposure (Ghorbel et al. 2015).

The fish liver is especially relevant to the immune toxicity of water pollutants, as shown by liver histopathological alterations and changes of liver-related biochemical markers after arsenite exposure in this study and by others (Saïdi et al. 2013; Benhamed et al. 2016; Webster et al. 2017). Heavy metals exposure has the ability to alter the immune response in different ways. This could be attributed to many factors associated with the heavy metal mode of action. For example, As can modulate gene expression by modifying the cellular redox status which leads to an imbalance of pro/antioxidant species with subsequent tissue damage and the initiation of inflammation (Lantz and Hays 2006). Moreover, As can influence cytokines expression by affecting components of upstream signal transduction pathways. Kinases such as protein kinase C (PKC), mitogen activated protein kinases (MAPKs), and IκB kinase (IκK) can be modulated by As exposure. Activation of these kinases is known to activate the transcription factor NF-κB, which in turn controls cytokine expression (Cobbina et al. 2015). On the other hand, Ch contains a large quantity of carotenoids that act as antioxidants leading to the reduction of reactive oxygen species (ROS) under oxidative stress, in turn expressing a higher level of NO and improving endothelial function (Hamias et al. 2018; Zahran and Risha 2014). Furthermore, β-carotene can inhibit NF-κB activity and suppress the expression of pro-inflammatory genes; indicating that ROS and inflammatory gene expression are interconnected through the NF-κB signaling pathway (Hamias et al. 2018; Soontornchaiboon et al. 2012). These results suggested the potential protective and therapeutic roles of Ch supplemented diets in regulation of cellular redox status, attenuation of inflammation and improvement of endothelial function.

As induced severe histopathological changes in Nile tilapia gills, livers and head kidneys. Gills of Nile tilapia exposed to arsenic showed hyperplasia, congestion and lamellar fusion. These results were in accordance with other previous studies (Abdel-Tawwab et al. 2007; Simonato et al. 2008; van Heerden et al. 2004). Lesions reported in gills may affect oxygen consumption and disrupt osmoregulatory function. These alterations may be due to the increase in cellular metabolism leading to an imbalance of osmotic regulation by impairing ionic active transportation (Mazon et al. 2002) and may be attributed to the direct responses induced by the action of the irritants as well (Kan et al. 2012). As the main site of detoxification, liver is the most sensitive organ to xenobiotics (Crestani et al. 2007). In the present study, intense degenerative changes in liver were seen in the arsenite exposed group Ch0+As, whilst the degeneration was observed to a lesser extent after Ch supplementation, especially in the Ch10+As group. These results are inconsistent with what had been described by Camargo and Martinez (2007) in *Prochilodus lineatus*, subjected to in situ tests along the upper reaches of Cambé stream. Additionally, these induced alterations suggested metabolic damage owing to As^{3+} exposure (Pacheco and Santos 2002). Several pathological changes were induced in kidneys exposed to As^{3+}, such as hematopoietic tissue necrosis and sinusoidal congestion. Similar results were observed in *Astyanax altiparanae* (Silva and Martinez 2007) and *Oreochromis mossambicus* (Ahmed et al. 2013). Similarly, histopathological alterations were reported in liver and gills of *Odonthestes bonariensis* inhabiting Chasicó Lake in the southwest of Buenos Aires Province, a lake known to have a high As concentration (Puntoriero et al. 2018). Our findings demonstrate that fish fed with a diet supplemented with *Chlorella* exhibited reduced histopathological alterations to some extent. Similar results were seen in *Oreochromis niloticus* fed *T. laurifolia* leaf extract and exposed to lead (II) nitrate (Palipoch et al. 2011). In the same context, cyclophosphamide administration in mice induced different pathological lesions in the form of cellular fibrosis, necrosis, and disordered cell arrangements, whereas supplementing Ch at 12 and 24% revealed normal cell appearance and minor fibrosis (Cheng et al. 2017).

Consistently, our results showed that As^{3+} exposed fish showed much greater damage in comparison to groups fed Ch diets, especially the Ch10+As group, which showed the best reduction in the detected damage in the examined organs and decreased As^{3+} induced tissue alterations due to its anti-inflammatory properties and its ability to maintain healthy endothelial function.

Chlorella dietary supplementation has great benefits of alleviating such toxic effects induced by As^{3+} exposure, including stabilization of the biochemical parameters and cytokine gene expression, and the reduction of induced histopathological alteration. Microalgae such as *Chlorella* spp. have major uses in aquaculture, owing to their high contents of proteins, fatty acids of the ϖ3 and ϖ6 types, and many essential vitamins, minerals and antioxidant substances, in addition to its immunostimulating properties (Reyes-Becerril et al. 2013). In the same context, *Chlorella* had a preferable action in inhibiting lipid peroxidation in comparison to glutathione and had antioxidant properties (Bengwayan et al. 2010). *Chlorella* has a great role in immune response via augmentation of macrophage activity (Hasegawa et al. 1997; Liu et al. 2006). Similarly, other studies evaluated the benefits of using microalgae against xenobiotic exposure in common carp (Pugazhendy 2012), and in mice (Queiroz et al. 2003). Similar results were seen in previous studies emphasizing the role of using dietary *Chlorella* (Andrews et al. 2009; Mason 2001; Suhendrayatna et al. 1999). Together with other previously documented studies relating to the growth promoting abilities of Ch, we can suggest the possibility of combined use of microalgae for aquaculture water treatment and growth promotion. Furthermore, Ch has proved its anti-tumor and anti-inflammatory properties through improving healthy endothelial function leading to therapeutic control of induced inflammatory diseases. Thus, Ch as GRAS is consumed for medical treatment in humans as well (Hamias et al. 2018).

To conclude, our findings have clearly demonstrated the deleterious effects of As^{3+} exposure on Nile tilapia and the potential protective and therapeutic roles of *Chlorella* supplemented diets. The Ch diet supplementation reduced the histopathological alterations in different tissues, maintaining the serum biochemical parameters, and reduced the hepatic immune gene expression in Nile tilapia exposed to sodium arsenite. Therefore, our previous and current findings represent a comprehensive study of As^{3+} induced toxicity in Nile tilapia, and show a substantial role of *Chlorella* as a future biotech tool in the advancement of green innovation for a sustainable aquaculture industry.

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