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Cytotoxic and genotoxic effects of arsenic on erythrocytes of *Oryzias latipes*: Bioremediation using *Spirulina platensis*

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ABSTRACT

Background: Exposure to the environmental pollutants poses a serious threat to aquatic organism. The arsenic exposure in fish increases the risk of developing serious alterations from embryo to adult.
Objectives: The present investigation was done to study the toxic effects of heavy metal arsenic [As(III)] on medaka (*Oryzias latipes*). Morphological alterations, apoptosis, nuclear abnormalities, and genotoxic biomarkers in erythrocytes were used to determine the stress caused by arsenic (As) exposure.
Methods: Medaka was exposed to As for 15 days at two toxic sublethal concentrations (7 ppm and 10 ppm) in combination with *Spirulina platensis* (SP) treatment as antioxidant algae at 200 mg/L.
Results: Results were consistent with a previous study results on tilapia. Exposure of medaka to As resulted in a dose-dependent increase in most the biomarkers used in the current study. Fish exposed to10 ppm As showed highest level of DNA damage. For the first time to our knowledge, using SP to counter the As toxicity in medaka, DNA damage restored to control levels.
Conclusion: Accordingly, those results suggests that SP can protect medaka in aquaculture against As-induced damage by its ability as reactive oxygen species (ROS) reducer, antioxidant role, and DNA damage scavenger.

1. Introduction

Environmental chemical pollution with heavy metals, as a result of increasing industrialization, represents a major problem worldwide. The toxicity of environmental heavy metals to fish has been studied. Excess levels of heavy metals in water cause severe toxicity in aquatic environments and their fauna [1–3]. One of the highly toxic heavy metals is arsenic (As) and its release in the aquatic environment is due to the natural and anthropogenic activities [4]. The inorganic form of trivalent arsenite [As (III)] is more toxic up to seven-fold fish than As (V) [5] and organic forms [6]. Exposure of fish to arsenic causes many effects, including survival reduction, growth retardation, size reduction, hemato-biological alterations, hormonal disruption, histopathological alterations, embryotoxicity, genotoxicity, and carcinogenic effects and other diseases [3,7–10].

Arsenic induces toxicological effects in aquatic life forms, including fish inhabiting contaminated environments which accumulate high levels of arsenic. Their consumption lead to many health risks to humans such as cancer and neurological disturbances [11]. It is widely known that arsenic induces apoptosis and necrosis in fish in concentration-time dependent style [7]. Also, As exposure leads to DNA damage, alteration in mitochondrial membrane, and ROS production [12]. Most studies recorded the toxicity of arsenic derivatives in mammalian cells [13,14] and aquatic species, including fish [5,7,10,15–19], where the use of phytoremediation is limited [7,20].

Recently, phytoremediation is one of the most desirable technologies for removal of environmental pollutants or detoxification, and uses plants, including algae, to render heavy metals harmless [7,21–25]. Microalgae were effective in hyperaccumulation of heavy metals because they contain a diverse class of bioactive compounds [26–28]. Starting from this important point, this study was carried out to investigate the phytoremediation of As using microalgae.

Spirulina platensis (SP) possess great heavy-metal absorption capacity [7,22] and are able to activate a definite set of biochemical and physiological processes to counter the toxic action of environmental contaminants in fish [7,22]. Currently, the chelating properties of SP that counter the arsenic-induced genotoxic effects of medaka have yet to be reported, to our knowledge. Medaka (*Oryzias latipes*) was used in extensive research and are considered an excellent model for toxicology [29,30] because of their sensitivity of stressors such as γ-IR [31,32],

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UVA [33,34], and chemical toxicants [15,35]. Fish hemato-biochemical alterations were used recently as biomarkers for metal pollution conditions and water quality [36–38]. Erythrocytes of *O. latipes* were used to study the cytotoxicity and genotoxicity of UVA, γ-IR, and 4-nonylphenol [33–35,39,40]. Therefore, this investigation aimed to study the ameliorative role of SP against cellular and genotoxic alterations in erythrocytes of medaka and their ability to absorb arsenic bioaccumulation.

2. Materials and methods

2.1. Medaka

In the present study, sexually female mature wild-type [WT; (Hd-rR)] medaka (*O. latipes;* 250–270 mg and 2.5–3.0 cm body length) were supplied by NBRP and maintained as closed colonies in the University of Tokyo. All fish were considered as healthy on the basis of an external examination for any signs of abnormalities or infestation. The fish were kept in a 5 L glass aquarium, aerated, and filtered dechlorinated water. Fish acclimated for two weeks to laboratory conditions at 26–28 °C under a 14 h light/10 h dark cycle. Fish feed on powdered food (Tetramin; Tetra Werke Co., Melle, Germany) or brine shrimp (*Artemia franciscana*) three times per day.

2.2. Arsenic (III) oxide and SP

Arsenic (III) oxide was purchased from Sigma-Aldrich, Tokyo, Japan (Cat. No. A1010, Lot. No. BCBS4599 V, and purity 99.9%). Spirulina tablets 100% were purchased from the Japan Algae Co., Ltd. (Tokyo, Japan) (Lot No, 3009), and freshly prepared suspension (200 mg/L) in water was used.

2.3. Experimental setup

The acclimatized fish were separated into five treatment groups (control, 7 ppm As (III) exposure, 7 ppm As (III) exposure with 200 mg/L SP, 10 ppm As (III) exposure, and 10 ppm As (III) exposure with 200 mg/L SP with six fish per group. In the present study, arsenic concentrations were chosen as environmentally relevant concentrations according to the literature [7,8]. In each treatment, the fish were placed into 1 L aquaria (3 samples per tank, two replicates). As (III) stock solution (1000 mg/L) was prepared to obtain nominal waterborne As concentrations of 7 ppm and 10 ppm. To ensure the stability of the nominal exposures and to decrease the impact of food on the experiment, the tank contents were thoroughly renewed 1 h after feeding every day. All exposure conditions were stable as the adaption conditions. After two weeks of exposure to treatment, fish were sampled before feeding, and anesthetized with ice-cold water [41]. Blood was collected immediately for blood smearing, and muscles stored at -80 °C until As residue analysis. During the experiment, physicochemical parameters were assessed at intervals and reported in Table 1. All experiments were conducted at the Tokyo University and Toyo University according to protocols approved (Permit No. C-14-02).

2.4. Erythron profile and apoptosis detection

Six blood smears were immediately prepared, dried, fixed, and stained with May-Grünwald solution, followed by Giemsa as reported [42]. Preparation and staining were done by the same person. Slides were selected based on staining quality, coded, randomized, and scored by a person blinded to the treatment. In each group, 12,000 cells (minimum of 3000 per slide) were examined with an oil immersion lens at magnification of 100 × objective with a 10 × eyepiece using microscope with a digital color camera (BX50; Olympus, Tokyo, Japan) [43]. Published criteria for identifying cytological and nuclear abnormalities including micronucleus (MN) were followed strictly according to Schmidt [44] and Carrasco et al. [43] to ensure accurate scoring. Apoptosis using acridine orange stain (Cat. No. A1031; Life Technologies, Carlsbad, CA) was recorded according to a modified protocol [34] and observed using a fluorescence microscope with a digital color still camera (DP-70; Olympus).

2.5. Genotoxic biomarkers

Three µl of blood was collected (caudal vein cut) from the control and As-treated fish. To stop endogenous DNA damage and to inhibit DNA repair in the unfixed cells, blood sample was placed on ice immediately [45]. According to our previous work [40], viability of cells more than 90% were considered for the analysis.

A neutral comet analysis was conducted to observe DNA damage, using protocol described previously [46] with minor modifications [40]. Sixty cells (per slide) were analyzed under a fluorescence microscope equipped with a digital still camera (DP-70; Olympus). CASP software was used for comet analysis and tail moment score calculation [47].

The γ-H2AX immunostaining to confirm DNA damage was described previously with minor modifications [40,48]. Finally stained RBCs mounted on a new glass slide examined and fluorescence images taken using a fluorescence microscope (IX-81; Olympus) equipped with a digital still camera (DS Ri1; Nikon, Tokyo, Japan).

2.6. As residue analysis

Two µL of blood was collected and 0.2 g of muscles of exposed and control medaka and 1 mL of filtered water (after As exposure, water samples were collected from exposure tanks in clean falcon tubes and then filtered using syringe (Cat. No. SS-10SZ, Terumo) and disposable membrane filter (Cat. No. 28HP045AN, Advantec). These were added to 2.0 mL of 1.0 (v/v) % ultrapure nitric acid (Cat. No. 28163-5B, Kanto Chemical Co.) in a 50-mL Teflon beaker (Cat. No. 33431097, AS One) and then mixed well. The beaker was wrapped in cellophane film to prevent contamination and incubated for 12 h. The cellophane film was then removed and the mixture heated using a hot plate (Cat. No. CRC-A300, Tiger) at 110 °C until just before going dry, and then cooled to room temperature. Then added 2.0 mL of nitric acid to the beaker and then mixed, heated, and cooled again. Add 2.0 mL of nitric acid plus 0.5 mL of hydrogen peroxide (Cat. No. 18084-1B, Kanto Chemical Co.) to the beaker, then mixed, heated, and cooled it. Dissolve the residue in

Table 1
Physicochemical parameters as mean ± SD (range) after exposure of adult female medaka (*Oryzias latipes)* to different doses of As and treatment with *Spirulina platensis*.

Treatments Parameters	Control	As 7 ppm	As 7 ppm + SP	As 10 ppm	As 10 ppm + SP
Dissolved Oxygen (mg/l)	7.6 ± 1.5 (5.9- **8.7**)[a]	7.8 ± 1.6 (**5.9- 8.8**)[a]	8.8 ± 1.4 (7.8- 10.4)[a]	8.4 ± 0.31 (8.2 - 8.79)[a]	6.0 ± 1.4 (4.6 -7.4)[a]
pH	7.2 ± 0.3 (**6.8**-7.4)[a]	7.2 ± 0.5 (**6.6**-7.5)[ab]	7.8 ± **1.1** (**6.7**-8.8)[ab]	7.2 ± 0.6(6.6-7.6)[b]	6.8 ± 0.4 (**6.4**-7.0)[b]
Temperature ºC	24.7 ± 0.1 (24.6-24.8)[a]	25.1 ± 0.2 (24.9- **25.3**)[a]	25.5 ± 0.2 (**25.3**-25.7)[a]	25.6 ± 0.2 (25.5-25.8)[a]	25.6 ± 0.2 (25.5-25.8)[a]
Conductivity (µS/cm)	20.6 ± 14.1 (7.1-35.2)[a]	21.7 ± 16.1 (7.1-39)[a]	49.6 ± 25.9 (20.6-70.4)[b]	23.8 ± 4.6 (19.6-28.7)[a]	84.9 ± 41.8 (36.7-109.1)[b]

[*] Different letters in subscript indicate a significant difference at $p \leq 0.05$.

4.0 mL of 1.0 (v/v) % nitric acid. Filter the dissolved sample into 15 mL of centrifuge tube using a glass fiber filter (Cat. No. 60219706, Advantec) fitted with funnel. Repeating the dissolution and filtration twice, adjusted the final volume to 12.0 mL. Finally, measured the metal concentration of the filtered sample using ICP-MS (NexION-300D; PerkinElmer, Walthman, MA) according to the manufacturer's guide [49].

2.7. Statistical analysis

The mean, standard deviation, and range were calculated. Differences between groups were estimated using a one-way analysis of variance and Dunnett post hoc tests at the 0.05 significance level with SPSS software [50], treating one as control to compare it with all the other groups.

3. Results

3.1. Behavioral change and physicochemical parameters

During the 15-day exposure, all fish in all treatments were alive and feeding normally, with no observable effect *de visu* on the extent of swimming, activity, and body color, indicating all fish were apparently healthy. The mean values of tested water qualities are reported in Table 1, where some significant differences were observed.

3.2. Erythron profile and frequencies of altered, micronucleated, and apoptotic erythrocytes

Fig. 1a shows light micrograph of blood smear of control group demonstrates the normal structure of erythrocytes, as described before by Sayed et al., (2014). Exposure of medaka to As (III) induced abnormal erythrocytes. The major alterations of the exposed fish (Fig. 1b-i) were the appearance of bilobed-cell (Bilc), micronucleated cells (Mn), acanthocytes with eccentric nuclei (Ac with Ecn), schistocytes (Sh), amoebocytes (Am), spindle-shaped cells (Sp), hemolyzed cells (Hc), crenated cells (Cr), blebbed nuclei (Bn), sickle cells (Sk), dividing cells (Dic), ovalocytes (Ov), cuboidal cells (Cc), keratocytes (Ke), tear-drop cells (Tr), fragmented nuclei (Fn), microcytes (Mc), and elliptocytes (El). Some of these alterations were observed for the first time after As (III) exposure in medaka fish, such as spindle-shaped cells, dividing cells, ovalocytes, cuboidal cells, keratocytes, fragmented nuclei, microcytes, and elliptocytes.

Table 2 and Fig. 2 show the frequencies of altered, micronucleacted (MN), and apoptotic erythrocytes of the medaka exposed to As (III). At all As doses tested the frequency of alterations including nuclear abnormalities, was the highest at 10 ppm As (III) exposure. As shown in Table 2, MN increased in the exposed groups compared with the control and the MN percentage increased significantly with increase of the As (III) dose. Compared to control, a significant increase in the apoptotic erythrocytes in fish exposed to As (III) was observed with the highest frequency in the fish exposed to 10 ppm As.

3.3. Genotoxic biomarkers for DNA damage

A bioindicator of the dose-dependent DNA damage in erythrocytes after As (III) exposure is γ-H2AX foci formation, where it was observed in control and treated groups (Fig. 3). The number of foci per nucleus was significantly higher after As (III) exposure compared with control and other fish treated with SP. The average number of γ-H2AX foci (n = 6) was 3.98 ± 1.41, 11.52 ± 2.89, 7.72 ± 1.84, 18.12 ± 3.73, and 10.12 ± 2.92 for control, As (7 ppm), As (7 ppm + SP), As (10 ppm), and As (10 ppm + SP), respectively (Table 2). The highest number of γ-H2AX foci was observed at 10 ppm As (III) exposure compared to control. The increase of γ-H2AX foci along the As (III) concentrations used suggesting that 15 days is dose-dependent in genotoxicity on erythrocytes of medaka.

Tail moment per cell was recorded after As (III) exposure and compared with the respective control and other fish treated with SP (Fig. 3). The percentage of tail moment was 4.31 ± 3.76%, 11.88 ± 5.26%, 6.95 ± 3.1%, 16.20 ± 5.33 and 8.97 ± 6.38% for control, As (7 ppm), As (7 ppm + SP), As (10 ppm), and As (10 ppm + SP) respectively (Table 2). However, the tail moment increased significantly in As (III) (7 ppm), As (10 ppm), and As (10 ppm + SP) compared to control fish. Moreover, the results in the As (III) treated groups showed the dose-dependent induction of DNA damage after arsenic treatment, with high potential of SP to reduce this damage.

3.4. Arsenic bioaccumulation in tested water, and blood and muscles of fish

Table 2 shows that As concentrations remained stable in the tested water and in the blood and muscles of the control fish while in the As (III) exposed groups, As concentrations in the tested water and in the blood and muscles increased gradually during the exposure period. In tested water, As bioaccumulation was 7.17 ppm and 13.81 ppm of the total As (III) exposure in 7 ppm and 10 ppm As exposed groups, respectively, while the bioaccumulation of As (III) was 3.86 ppm and 9.72 ppm of the total As (III) exposure in 7 ppm + SP and 10 ppm As + SP exposed groups, respectively. This trend of SP-chelating ability was recorded in blood and muscles of medaka after As exposure.

4. Discussion

The arsenic entered the aquatic environment is of natural origin, in concentrations of about 1.9 μg/L was reported in Japanese river water [51], although this concentration increased recently in 2015 to reach about 38.9 ppm in water and 55.2 ppm in sediments of rivers [52]. While recent literature studied the hematological and molecular effects of As on fish [53–55], only limited studies on DNA damage were available. Therefore, this investigation has been conducted to report the observations of cytotoxic and genotoxic alterations of As-induced DNA damage stress in medaka.

In the present study, medaka (*O. latipes)* exhibited no observable behavioral effects *de visu* because of exposure to sublethal concentrations of As. This is consistent with a recent study by Chen et al., [15], who reported that freshwater *O. mekongensis* become more tolerant to effects of As (V) after exposure to As (III), indicating the resistance acquire of fish after exposure to high concentrations of As [56]. Whereas other behavioral changes, such as excessive secretion of mucus, lateral swimming, and equilibrium loss were observed especially from a high dose of arsenic in *Anabas testudineus* (250 ppm) and in *Danio rerio* (5 and 15 ppm), respectively [57,58]. Although As-induced genotoxic effect in fish, to our knowledge this is the first in vivo study to have found apoptosis and DNA damage in erythrocytes induced by sublethal concentrations of As in Japanese medaka [10]. Sublethal As-exposure affects the morphological and nuclear structure of erythrocytes which accordingly make the fish adaptive immunity delayed [59] because As exposure has an influence on the immune organs, head kidney, and spleen [60], which are major organs for erythrocyte production in fish.

Arsenic induces DNA damage and formation of MN in catfish and Tilapia [7,61]. Also, production of ROS after As exposure caused apoptosis in both cell lines and Tilapia [7,62]. This mechanism can help to explain the concentration-dependent cytotoxic alterations in erythrocytes of medaka after As (III) exposure. These results are consistent with those of others, who reported that inorganic As effects include enzymes and gene alterations [63]; high concentrations (3 ppm, 28 ppm, and 56 ppm) of As cause cell death in liver cells of *Oreochromis mossambicus* [64]; As (III) induces apoptosis and necrosis-mediated cell death [65], and induces apoptosis, micronuclei, and erythrocyte alterations in tilapia [7]. Arsenite may induce apoptosis because it induce

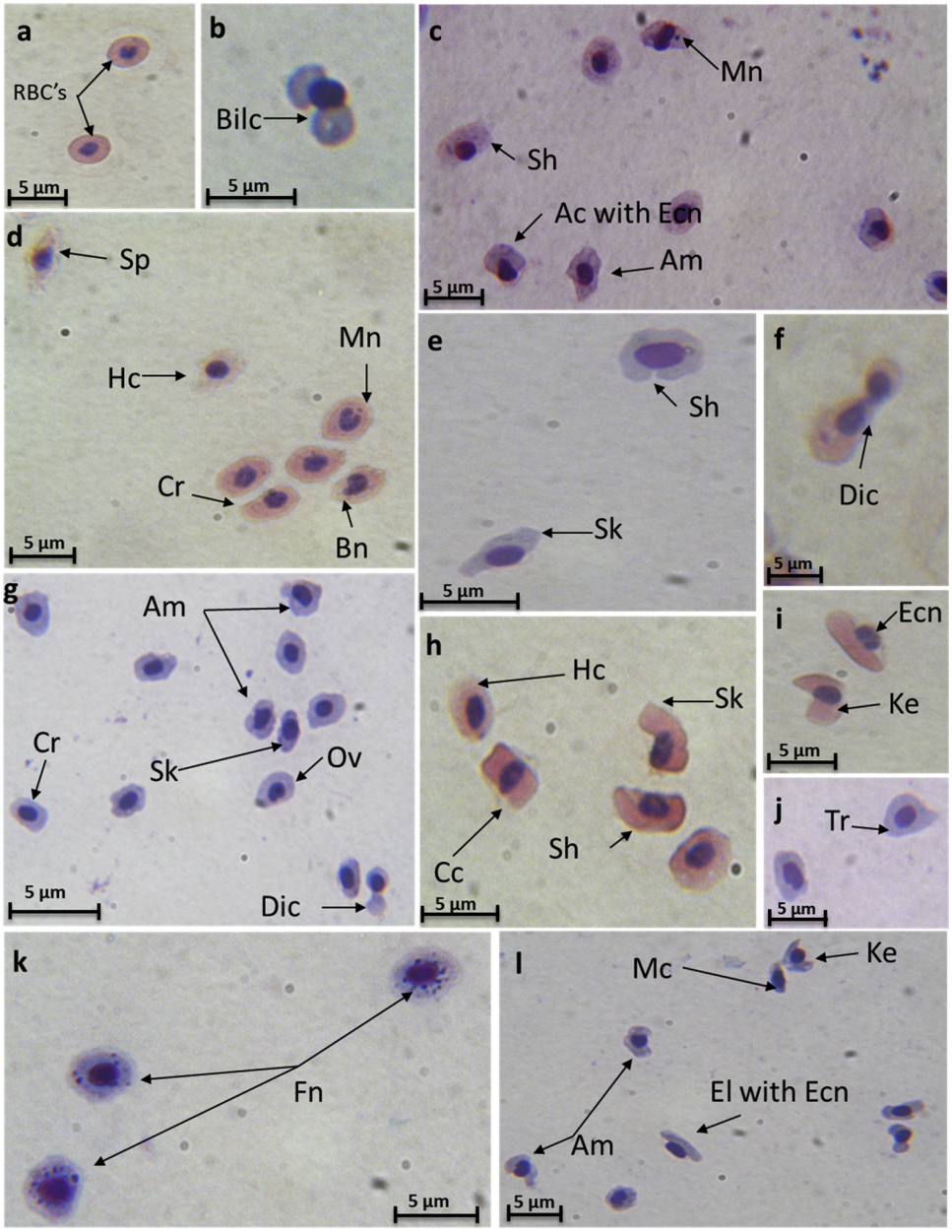

Fig. 1. Light micrographs of Giemsa-stained blood smears showing examples of arsenic-induced morphological alterations and nuclear abnormalities in erythrocytes of medaka (*Oryzias latipes)* exposed to As treatment in vivo, (a) control fish showing normal red blood cells (RBCs); (b–g) fish exposed to 7 ppm As showing Bilc; bilobed cell, Mn; micronucleated cell, Ac with Ecn; acanthocyte with eccentric nucleus, Sh; schistocyte, Am; amoebocyte, Sp; spindle-shaped cell, Hc; hemolyzed cell, Cr; crenated cell, Bn; blebbed nucleus, Sk; sickled cell, Dic, dividing cell; Ov; ovalocyte, Cc; cuboidal cell, Ke; keratocyte, Tr; tear-drop cell, Fn; fragmented nucleus, Mc; microcyte, and El; ellipocyte.

Table 2
Genotoxic biomarkers and As concentration as mean ± SD (range) after exposure of adult female medaka (*Oryzias latipes)* of wild-type (WT) (Hd-rR) to different doses of As and treatment with *Spirulina platensis*.

Treatments Parameters	Control (n = 6)	As 7 ppm (n = 6)	As 7 ppm + SP (n = 6)	As 10 ppm (n = 6)	As 10 ppm + SP (n = 6)
Altered erythrocytes (%)	1.3 ± 1.2 (0-4)^{a}*	14.2 ± 2.4 (11-19)^{b}	4.5 ± 2.5 (1-11)^{a}	46.2 ± 9.7 (24-72)^{c}	17.0 ± 7.2 (10-42)^{b}
Apoptotic cells (%)	2.0 ± 1.2 (0-5)^{a}	5.1 ± 1.2 (2-7)^{c}	2.0 ± 0.9 (1-4)^{d}	24.7 ± 1.8 (21-32)^{a}	11.2 ± 3.2 (2-19)^{b}
MN (%)	0.0 ± 0.0 (0-0)^{a}	4.8 ± 4.8 (3-8)^{b}	3.7 ± 1.7 (0-8)^{c}	7.8 ± 2.4 (1-11)^{d}	4.4 ± 1.2 (2-7)^{bc}
γ -H2AX foci per cell**	4.0 ± 1.4 (0 - 7)^{a}	11.5 ± 2.9 (1 - 15)^{c}	7.7 ± 1.8 (3 - 14)^{b}	18.1 ± 3.7 (12 - 33)^{d}	10.1 ± 2.9 (4 - 18)^{c}
Tail moment score **	4.3 ± 3.8 (0.00 - 13.99)^{a}	11.9 ± 5.3 (0.52 -23.73)^{b}	7.0 ± 3.1 (0.07 – 13.42)^{a}	16.2 ± 5.3 (6.36 – 39.70)^{a}	9.0 ± 6.4 (0.73 – 33.87)^{b}
As residue in blood (ng/g)	20.3 ± 16.4 (0-46)^{a}	278.5 ± 258.9 (40.4-691.37)^{ab}	65.7 ± 69.0 (4.94-189)^{a}	322.8 ± 131.2 (203.31-549.64)^{b}	225.9 ± 176.0 (83.2-555.8)^{ab}
As residue in muscle (ng/g)	305.0 ± 92.5 (235.97-485.61)^{a}	2213.3 ± 924.7 (1262.71-3537.88)^{b}	1763.0 ± 580.4 (992.70-2715.70)^{b}	2685.5 ± 1000.2 (2031.51-4652.18)^{b}	2415.1 ± 1006.9 (1371.78-4057.20)^{b}
As residue in water (mg/L)	0.0 ± 0.0 (00-00)^{a}	7.2 ± 0.3 (6.77-7.62)^{b}	3.9 ± 0.2 (3.48-4.12)^{c}	13.8 ± 1.6 (11.12-15.8)^{d}	9.7 ± 0.5 (9.14-10.48)^{e}

*Different letters in subscript indicate a significant difference at $p \leq 0.05$.
**Sixty cells were measured per group.

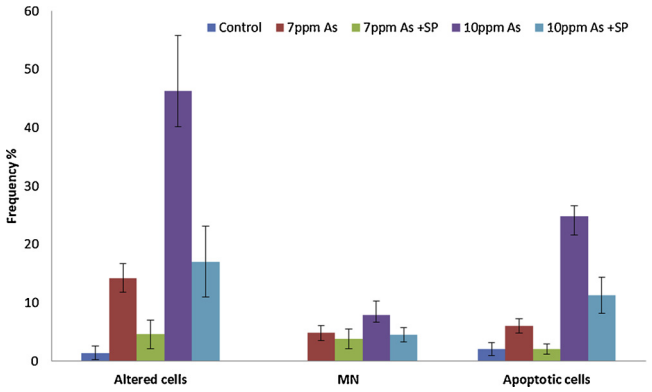

Fig. 2. Percentage of altered, apoptotic, and micronucleated erythrocytes (mean ± SD) after exposure to two doses of As (III) and SP per 100 cells of wild-type (WT) (Hd-rR) adult female medaka (*Oryzias latipes)*.

oxidative stress, many genetic alterations (DNA damage), disrupted cell division, large deletion mutations, chromosomal damage, aneuploidy, MN formation, DNA-protein cross linking, DNA fragmentation, cell cycle arrest, and sister chromatid exchange [7,61,63]. By contrast, dose-dependent increases in the induction of erythrocyte alterations, micronuclei, apoptosis, and γ-H2AX foci were in the present study. This is in agreement with Ramirez and Garcia [66], who suggested that DNA and protein damage is a result of As metabolism and oxidative stress, which in support of all the cellular damage in the present study. An increase in MN and nuclear abnormalities with duration of As treatment at sublethal concentrations in zebrafish (0.395 to 0.630 ppm) and in *Channa punctate* (6.936 ppm) were reported with an increase in micronucleus frequency [66,67]. Compared with tilapia (*O. nitoticus*), medaka showed a high level of MN, and altered and apoptotic erythrocytes [7]. Although As induced DNA damage in gills, liver, and blood tissues of fish examined by Ahmed et al. [18] and Datta et al. [68] after 14- and 30-days exposure, phytomedicinal protection against As toxicity in fish requires further study.

The modulatory role of SP using biomarkers of oxidative stress was reported to be induced at much higher As concentrations (10 ppm), is worth noting. This is in agreement with several other changes in fish induced by As exposure, including apoptosis (Roy and Bhattacharya, 2006) and micronuclei induction [7,66].

The use of SP improved genotoxic parameters tested in medaka exposed to arsenic, especially at the high dose (10 ppm) of sodium arsenite. This may be attributed to the presence of protein and carbohydrate in SP, which are considered excellent ligands for metal ions [69–72]. Extensive studies have evaluated the ameliorating properties

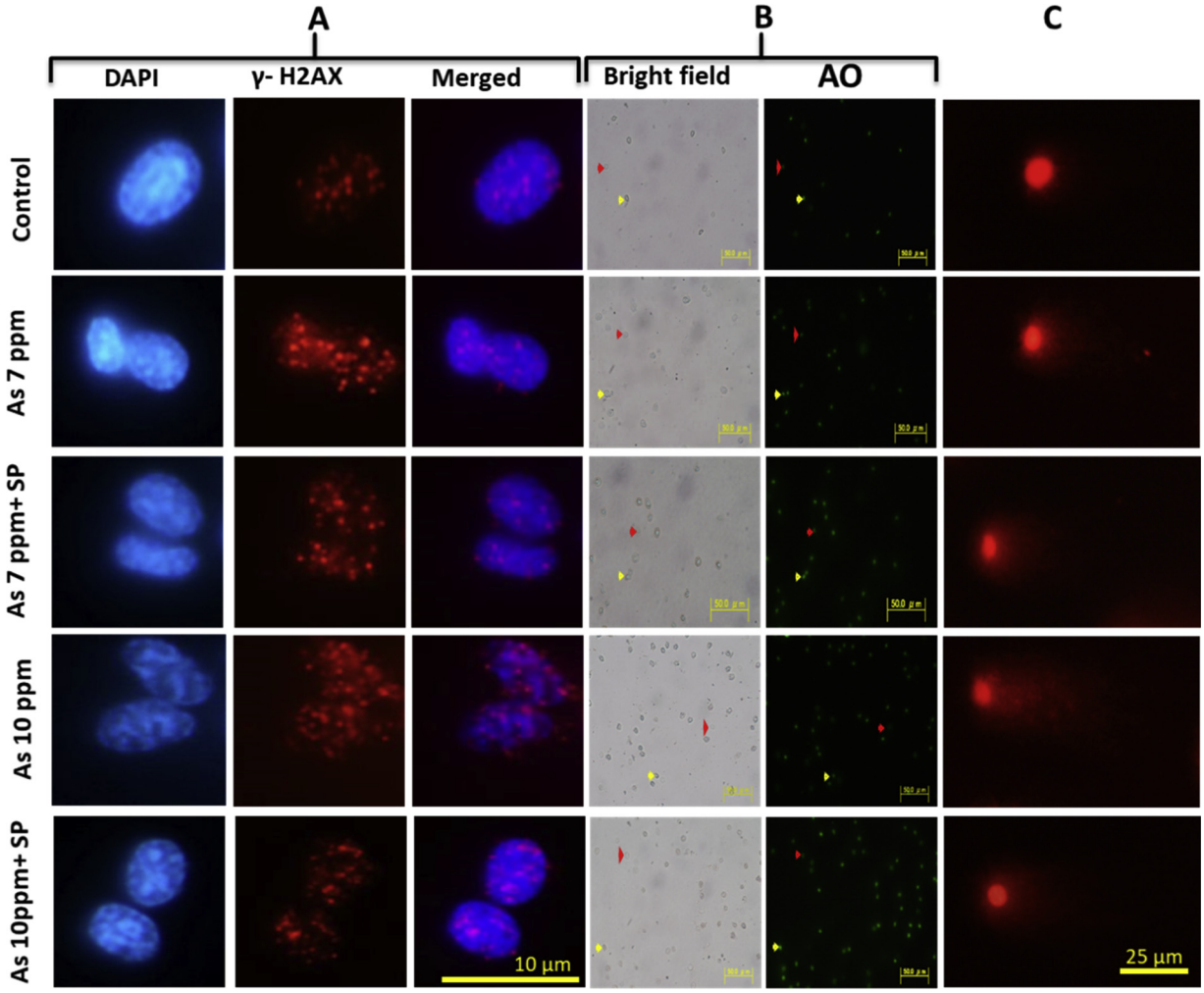

Fig. 3. Erythrocytes of sexually mature wild-type (WT) (Hd-rR) adult female medaka (*Oryzias latipes*) exposed to As (III) with SP treatment, showing (A) formation of γ-H2AX foci in controls and exposed groups, each panel shows DAPI-stained nuclei (blue), γ-H2AX (red), and merged images (scale bar = 10 µm); (B) apoptosis detection in cells stained with acridine orange (AO), where yellow arrowheads indicate apoptotic cells, red arrowheads indicate nonapoptotic cells; and (C) comet tails stained with ethidium bromide indicating DNA damage (scale bar = 25 µm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

of algae against heavy-metal and radiation effects [21,23,24,73–75]. In particular, microalgae possess several appreciable mechanisms to sequester heavy metal ions, and are considered promising biosorbents [76–78]. To date, little is known regarding the mechanism by which SP extracts can ameliorate the toxic damage induced by heavy metals in fish, while many studies show the protective role of SP [7,21,22,24]. A previous research concluded that SP supplementation, as an immune system inducer and growth factor in fish feed, improves hematological parameters in *Clarias gariepinus* [21]. SP has also been reported as an antioxidant, antiapoptotic, antigenotoxic, and lead-chelating agent in fish [7,22,24,79]. In the present work, As accumulated in the tested water, and in blood and muscles of fish during 15 days exposure, but decreased significantly after treatment with SP. The level of bioaccumulation increased steadily, and matched that found in previous studies [15,56]. Chen et al. [15], explained the bioaccumulation of As in *O. mekongensis* on the basis of toxicokinetics for the first time, attributing the reduced or balanced body As concentration to a potential mechanism for As acclimation, which requires further confirmation studies. Another mechanism of As tolerance may be the ability of fish to metabolize the toxic inorganic form of As to the less toxic organic forms of As [80,81]. As methylation as a strategy of fish to detoxify and acclimate to As was studied in *Tilapia mossambica*, *O. latipes*, and *O. mekongensis* [5,15,82]. Other mechanisms not so extensively studied are phytochelatins using microalgae [20], whereas the present results indicate for the first time to our knowledge that SP treatment can initiate various mechanisms to weaken As (III) toxicity in medaka. These strategies may be isolated or combined, for example, to decrease uptake of As from environmental water or to enhance the ability of medaka to excrete As from cells, synthesize metal-binding peptides, or increase biotransformation into organic As [56,80,83]. To determine the fate of the As accumulation in tissues, the current findings should be further supported by histopathological evidence in future studies.

5. Conclusion

According to the present study, medaka showed sensitivity to arsenic exposure compared to other fish species (Tilapia), reinforcing its characteristic as a toxicological model. *S. platensis* can be used widely as a chelating agent or detoxification factor for arsenic.

Acknowledgments

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