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RESEARCH ARTICLE

Role of Spirulina in mitigating hemato-toxicity in Swiss albino mice exposed to aluminum and aluminum fluoride

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Abstract Aluminum is ingested through foods, water, air, and even drugs. Its intake is potentiated further through foods and tea prepared in aluminum utensils and Al salt added in the drinking water for removal of suspended impurities and also fluoride in the affected areas. The ameliorating role of a blue green alga Spirulina is well documented to various pollutants in the animal models. We, therefore, examined its protective role (230 mg/kg body weight) on the hematology of male Swiss albino mice treated with aluminum (sub-acute = 78.4 mg/kg body weight for 7 days, sub-chronic = 7.8 mg/kg body weight for 90 days) and aluminum fluoride (sub-acute = 103 mg/kg body weight, sub-chronic = 21 mg/kg body weight), along with their recovery after 90 days of sub-chronic exposure. This study revealed significant reduction in the values of RBC (5–18 %), Hb (15–17 %), PCV (8–14 %), and platelets (26–36 %), and increase in WBC (54–124 %) in the treated mice, particularly after sub-acute exposure. Aluminum fluoride was comparatively more toxic than aluminum. Further, Spirulina supplement not only alleviated toxicity of test chemicals in Swiss albino mice but also led to their better recovery after withdrawal.

Keywords Swiss albino mice · Aluminum · Aluminum fluoride · Blood cells · Spirulina

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Introduction

Aluminum is ingested through foods, water, air, and even drugs. Its intake is potentiated further through foods and tea prepared in aluminum utensils and Al salt added in the drinking water for removal of suspended impurities and also fluoride in the affected areas (Gupta et al. 2009; Bignucolo et al. 2012). Interestingly, uptake of aluminum is potentiated in the presence of fluoride in the potable water (Dai et al. 1994).

Hematological study is an important diagnostic tool in medicine for disease diagnosis. It has also been reported valuable to monitor pollutant stress. Erythrocyte is one of the major target tissues for aluminum toxicities (Zhang et al. 2016). A decrease in Hb, MCV, MCH, MCHC, PCV, and ferritin (Turgut et al. 2004), disturbance in heme synthesis (Graczyk et al. 2000), and microcytic anemia (Becaria et al. 2002) has been reported. Aluminum fluoride complexes formed spontaneously in aqueous solutions containing fluoride and traces of aluminum ions alter structure and function of erythrocyte membrane (Suwalsky et al. 2004).

Aluminum, a pro-oxidant, forms free radicals both in vitro preparations and in vivo (Exley 2004). Aluminum fluoride sparingly soluble in water dissociates in to aluminum and fluoride ions (Kirk and Othmer 1980). Fluoride exposure also generates free radicals (Sharma et al. 2007). Antioxidants as diet supplements reversed aluminum and fluoride-induced toxicity (El-Demerdash 2004; Orta and Erkan 2014). Several plant species rich in antioxidants may therefore, be used as diet supplements to counter free radicals induced toxicity.

Spirulina is a blue green alga rich in protein, free amino acids, minerals (calcium, phosphorus, potassium and iron), antioxidants (β-carotene, total carotenoids, phycocyanin), essential fatty acids (linoleic and γ-linoleic acids), and vitamin

Table 1 The detailed layout of the experiment

Groups	Treatments	Dose (mg/kg body weight/day)	Number of mice	Autopsy
Diet: standard feed				
Group-1	Al^{+3}	78.4	10	8th day
(sub-acute)	AlF_3	103	10	8th day
	Control	Vehicle	10	8th day
Diet: standard feed + Spirulina (230 mg/kg b.wt./day)				
Group-2	Al^{+3}	78.4	10	8th day
(sub-acute)	AlF_3	103	10	8th day
	Control	Vehicle	10	8th day
Diet: standard feed				
Group-3	Al^{+3}	7.8	10	91st day
(sub-chronic)	AlF3	21	10	91st day
	Control	Vehicle	10	91st day
Diet: standard feed + Spirulina (230 mg/kg b.wt./day)				
Group-4	Al^{+3}	7.8	10	91st day
(sub-chronic)	AlF_3	21	10	91st day
	Control	Vehicle	10	91st day
Post-treatments: diet—standard feed				
Group-5	Al^{+3}	Withdrawal	10	181st day
(sub-chronic)	AlF_3		10	181st day
	Control		10	181st day
Post-treatments: diet—standard feed + Spirulina (230 mg/kg b.wt./day)				
Group-6	Al^{+3}	Withdrawal	10	181st day
(sub-chronic)	AlF_3		10	181st day
	Control		10	181st day

B complexes (Hemalatha et al. 2012; Abdelkhalek et al. 2015). It has been reported as a powerful antioxidant (Krishna Mohan et al. 2006, Huang and Zhang 2007); anti-inflammatory and immunomodulatory (Abdelkhalek et al. 2015); hepato, reno, and cardio protective (Ibrahim and Abdel-Daim 2015; Saber et al. 2015; Abdel-Daim et al. 2015, 2016; Yadav et al. 2015, 2016); prevent anemia (Hemalatha et al. 2012); and improve haemopoiesis (Sharma et al. 2013; El-Sabagh et al. 2014). Spirulina also modulated drugs (Krishna Mohan et al. 2006; Khan et al. 2006; Ibrahim and Abdel-Daim 2015) and heavy metals induced toxicity (El-Desoky et al. 2013; Elshazly et al. 2015).

This study was performed to explore ameliorative role of Spirulina on the hematology of Swiss albino mice exposed to aluminum and aluminum fluoride. Their recovery was also monitored after withdrawal of test chemicals and important findings made are reported in this communication.

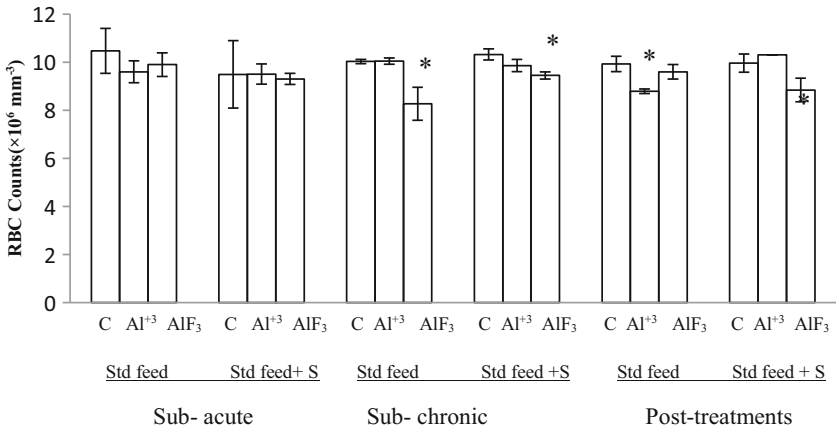

Fig. 1 RBC counts of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $^*p < 0.05$

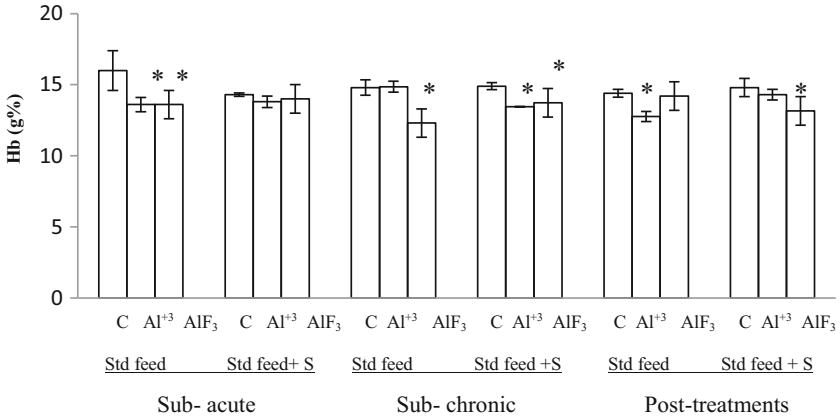

Fig. 2 Hemoglobin content of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$, $**p < 0.01$

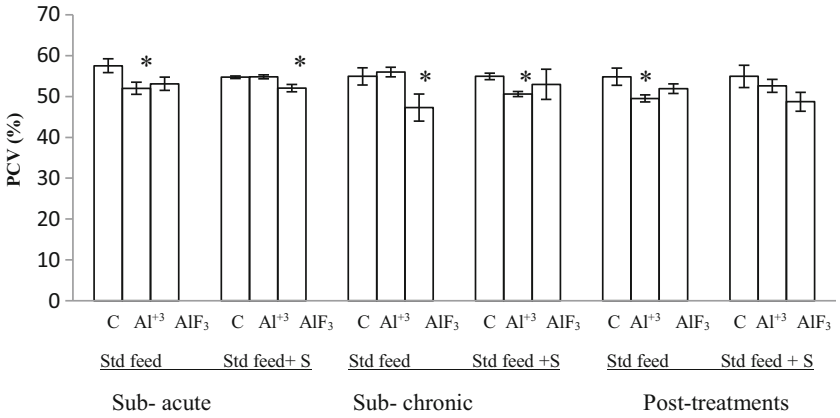

Fig. 3 Packed cell volume (PCV) of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$

Materials and methods

Animals and experiment design A total of 180 healthy young male mice (age = 75–80 days, weight = 30 ± 0.5 g) acclimatized for a week prior to entry in to experimental protocol were allotted randomly to six groups of 30 mice each. These were housed (five mice per cage) in polypropylene cages (50 cm (length) × 25 cm (width) × 15 cm (height)) kept in a well-ventilated animal house (Temperature 24 ± 3 °C; humidity = 40–60 %; 12 h light to dark cycle). All regulations of the Institutional Animal Ethical Committee (1678/GO/a/12/CPCSEA) were followed. Group-1, 3, and 5 received standard diet throughout the study period (hereafter referred to as standard feed groups in the text) whereas group 2, 4, and 6 were fed diet supplement Spirulina along with standard diet, 45 days prior to entry into experimental protocol until termination of study (hereafter referred to as Spirulina groups in the text, Table 1).

Based on reported LD_{50} values of test chemicals ($Al_2(SO_4)_3.16H_2O$, Merck Ltd., Mumbai, India, and AlF_3, Hi Media Laboratories Pvt. Ltd. Mumbai, India), their sub-acute and sub-chronic doses were calculated (Ondreicka et al. 1966; Lewis 1996). Test chemicals were dissolved in distilled water

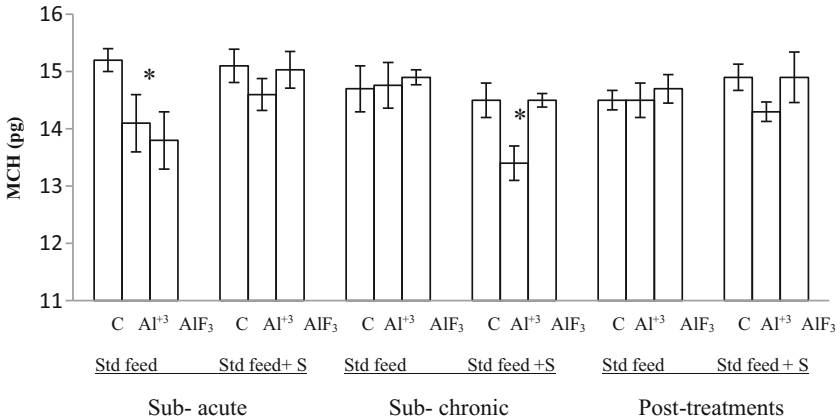

Fig. 4 Mean corpuscular hemoglobin (MCH) of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$

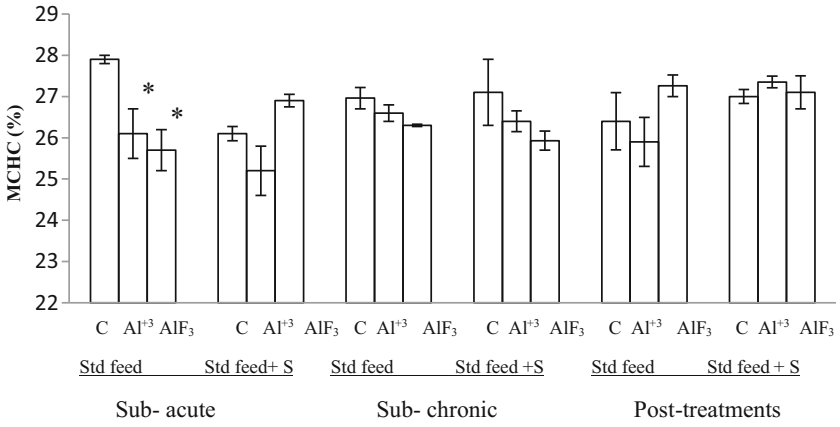

Fig. 5 Mean corpuscular hemoglobin concentration (MCHC) of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$

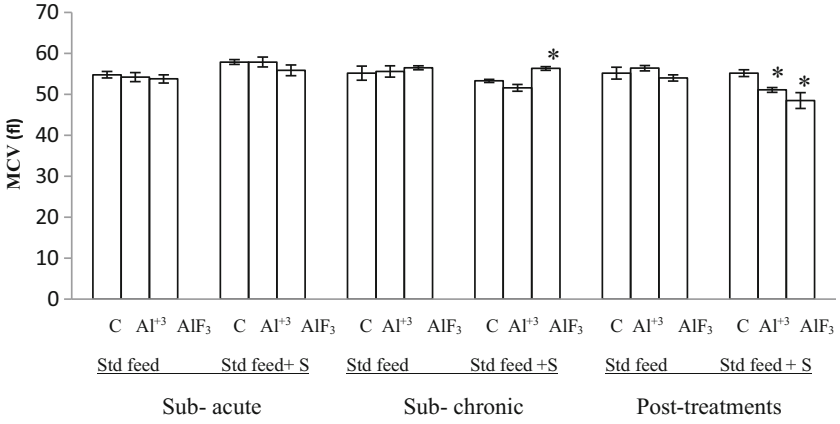

Fig. 6 Mean corpuscular volume (MCV) of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$

and mice were exposed through gavage (0.5 mL) to their sub-acute/sub-chronic doses (Table 1). Control mice received an equivalent amount of vehicle (distilled water) for the exposed period. The exposure of test chemicals to group-5 and 6 were withdrawn after 90 days, and thereafter mice were reared similar to controls. These are referred to as post-treated mice hereafter in the text.

All groups had free access to potable water (pH = 7.1; ER = 0.55 MΩ/cm; total hardness =198 mg/L; chlorides = 30 mg/L; fluoride = 0.9 mg/L; aluminum=nil) and standard laboratory diet (Ashirwad Ltd., Chandigarh, India) ad libitum. Animals were sacrificed by cervical dislocation at the termination of study.

Because AlF_3 forms a suspension in distilled water, care was taken to ensure complete delivery of its calculated dose. The suspension (3.5 mL) of sub-acute/sub-chronic dose of AlF_3 was prepared in marked vials (1–10) and 0.5 mL of this was administered to mice having the corresponding marking (1–10).

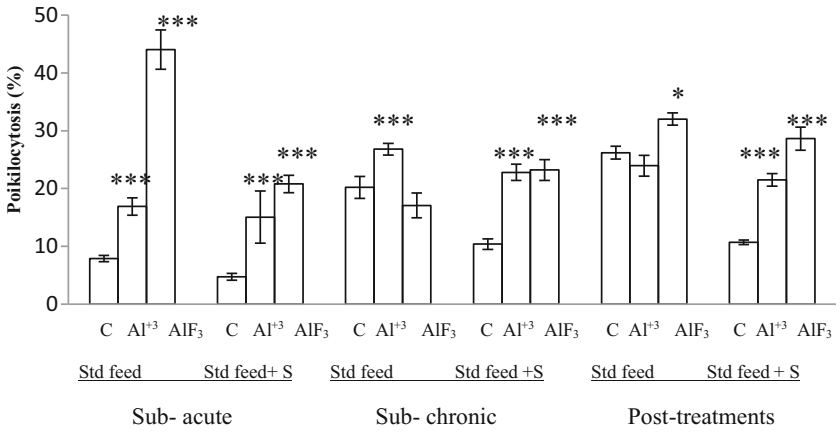

Fig. 7 Poikilocytosis (%) in RBC of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$, $***p < 0.0001$

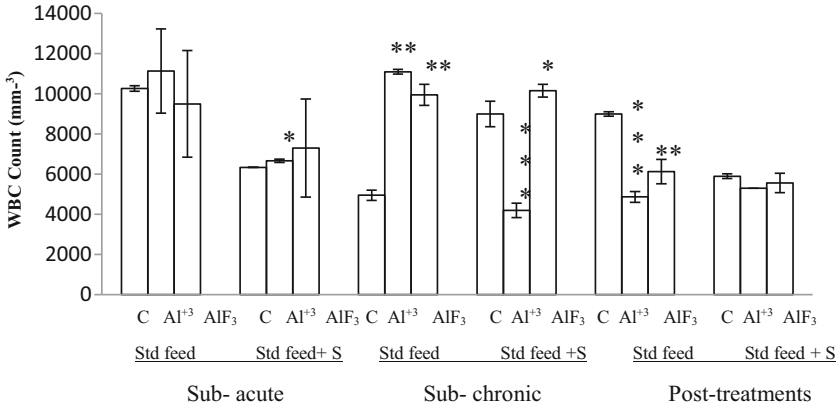

Fig. 8 WBC counts of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups. *Std* standard feed, *S* Spirulina. Significant at $*p < 0.05$, $**p < 0.01$, $***p < 0.0001$

Table 2 Differential leucocyte counts in controls, Al^{+3}, and AlF_3-treated mice (sub-acute) of standard feed and Spirulina groups

Leucocytes	Std. feed group			Spirulina group		
	Control	Al^{+3}	AlF_3	Control	Al^{+3}	AlF_3
Neutrophils	6.7 ± 3.8	23.7 ± 3.57**	22.2 ± 2.3***	9.3 ± 1.51	10.0 ± 2.17	17.3 ± 2.18**
Lymphocytes	39.9 ± 5.4	68.9 ± 4.4***	70.3 ± 2.1***	88.2 ± 1.34	85.5 ± 2.5	79.4 ± 1.94***
Monocytes	2.2 ± 0.4	6.1 ± 1.269**	5.8 ± 1.76	2.1 ± 0.35	2.8 ± 0.55	2.0 ± 0.516
Eosinophils	0.2 ± 0.4	1.0 ± 0.44	1.6 ± 0.718	0.1 ± 0.1	1.6 ± 0.45*	1.2 ± 0.70
Basophils	Nil	0.3 ± 0.21	0.1 ± 0.1	0.3 ± 0.21	0.1 ± 0.1	0.1 ± 0.1

Significant at $*p < 0.05$, $**p < 0.01$, and $***p < 0.0001$

Sunova capsule (from Dabur Ltd.) containing fine dark blue-green spray-dried powder of *Spirulina platensis* was the source of diet supplement to mice (230 mg/kg body weight, Yadav et al. 2015). Its fine suspension (0.5 mL/day/mouse) made in the distilled water was administered through gavage to Swiss albino mice.

Hematological analysis

Blood samples drawn from the heart of animals were stored separately in EDTA-coated vials. Hematological parameters analyzed using a Sysmex KX 21 cell counter were as follows: red blood cell (RBC) counts, white blood cell (WBC) counts, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet counts. Blood smears were prepared for differential leucocyte counts (Lee et al. 1999). Almost 200 erythrocytes were observed in 20 microscopic fields (10 × 100×) to quantify morphological abnormalities (Sharma et al. 2009).

Data analysis

Results are expressed as mean ± SEM. One way ANOVA was applied to find significant difference between values of various parameters recorded for control and treatments.

Table 3 Differential leucocyte counts in controls, Al^{+3}, and AlF_3-treated mice (sub-chronic) of standard feed and Spirulina groups

Leucocytes	Std. feed group			Spirulina group		
	Control	Al^{+3}	AlF_3	Control	Al^{+3}	AlF_3
Neutrophils	11.73 ± 1.7	8.8 ± 1.86	19.5 ± 2.12**	16.33 ± 1.89	23.2 ± 2.4*	11.5 ± 1.46*
Lymphocytes	36.9 ± 1.7	89.9 ± 2.19	77.8 ± 2.26**	83.2 ± 1.87	74.5 ± 2.28**	86.0 ± 1.95
Monocytes	1.13 ± 0.3	1.3 ± 0.49	2.6 ± 0.52**	0.27 ± 0.5	2.2 ± 0.8**	1.9 ± 0.57***
Eosinophils	0.07 ± 0.07	Nil	0.1 ± 0.1	0.13 ± 0.09	0.1 ± 0.1	6.6 ± 0.22
Basophils	0.13 ± 0.09	Nil	Nil	Nil	Nil	Nil

Significant at $*p < 0.05$, $**p < 0.01$, and $***p < 0.0001$

Table 4 Differential leucocyte counts in post-controls and Al^{+3}, and AlF_3 post-treated mice (sub-chronic) of standard feed and Spirulina groups

Leucocytes	Std. feed group			Spirulina group		
	Control	Al^{+3}	AlF_3	Control	Al^{+3}	AlF_3
Neutrophils	23.8 ± 4.3	20.25 ± 1.79	17.1 ± 1.43	15.8 ± 2.5	19.5 ± 3.36	15.1 ± 1.29
Lymphocytes	71.8 ± 4.3	77.38 ± 1.57	81.2 ± 1.39	78.5 ± 3.2	76.83 ± 4.16	79.11 ± 1.58
Monocytes	4.2 ± 0.77	2.2 ± 0.59*	1.6 ± 0.35*	5.6 ± 0.9	3.67 ± 0.99	5.78 ± 0.59
Eosinophils	0.82 ± 0.32	0.125 ± 0.12	0.1 ± 0.1	0.1 ± 0.1	Nil	Nil
Basophils	Nil	Nil	Nil	Nil	Nil	Nil

Significant at $*p < 0.05$

Results and discussion

The intoxication of test chemicals decreased RBC counts (5–18 %) and Hb (15–17 %) in the standard feed groups (Figs. 1 and 2). Other workers made similar findings in Al^{+3}-treated mice (Turgut et al. 2007; Milovanovic et al. 2012). The interference of aluminum with iron homeostasis blocks erythropoiesis (Pérez et al. 2001, 2005; Suwalsky et al. 2004) that decreases erythropoietin production (Celik et al. 2002). Aluminum exposure also induces eryptosis of erythrocytes (Vota et al. 2012). All these events decreased RBC counts and Hb content in the treated mice. The supplementation of Spirulina decreased toxic effects of test chemicals on RBC counts and Hb content, particularly in the sub-acute treatments. Phycocyanin pigment of Spirulina stimulates erythropoietin hormone production for hematopoiesis (Henrikson 1994) while higher content of iron and vitamins prevent anemia (Hemalatha et al. 2012). Erythropoietin also reduces eryptosis due to aluminum-induced oxidation stress (Vota et al. 2012). The supplementation of Spirulina thus protected anemia in Swiss albino mice. The recovery in RBC counts and Hb content was poor in the post-treated mice (Figs. 1 and 2). This may be related to retention of aluminum in the erythrocytes even at the time when its concentration in the plasma begins to fall (Milovanovic et al. 2012).

A reduction in PCV, MCH, and MCHC was found in Al^{+3} and AlF_3 treatments (standard feed groups=2–14 %, Spirulina groups=1–8 %; Figs. 3, 4, and 5). MCV values, however, differed little with controls (Fig. 6). Similar to present study, Turgut et al. (2007) reported reduction in values of MCV, Hb, RBC, Hct, MCH, and MCHC in aluminum-treated rats, being significant only for MCV. The reduction in values of RBC counts, Hb and their derived indices (MCV, MCH, and MCHC) suggests microcytic anemia (Becaria et al. 2002).

The percentage of poikilocytic RBCs was significantly higher in the treated mice of standard feed groups, particularly in AlF_3 treatments (Fig. 7). Other workers noted similar abnormalities in erythrocytes after Al^{+3} and AlF_3 toxicity (Vittori et al. 2002; Suwalsky et al. 2004). There are several reports indicating erythrocyte membrane to be one of the major target tissues for aluminum toxicity (Vittori et al. 2002; Suwalsky et al. 2004). Aluminum concentrates in the water lipid interface of membrane and interacts with the phosphates of the external hemilayer, thus diminishes the membrane external surface area (Thirunavukkarasu et al. 2011). It also induces lipid peroxidation and oxidative stress that damage molecular structure and fluidity of erythrocyte membrane (Bazzoni et al. 2005; Lukyanenko et al. 2013). Aluminum also disturbs erythroid cell maturation that may also induce morphological abnormality (Chmielnicka et al. 1993; Suwalsky et al. 2004). Thus, aluminum distorts both mature and young erythrocytes. Erythropoietin reduces aluminum-induced oxidative stress that decreased morphological abnormalities in the Spirulina groups (Vota et al. 2012).

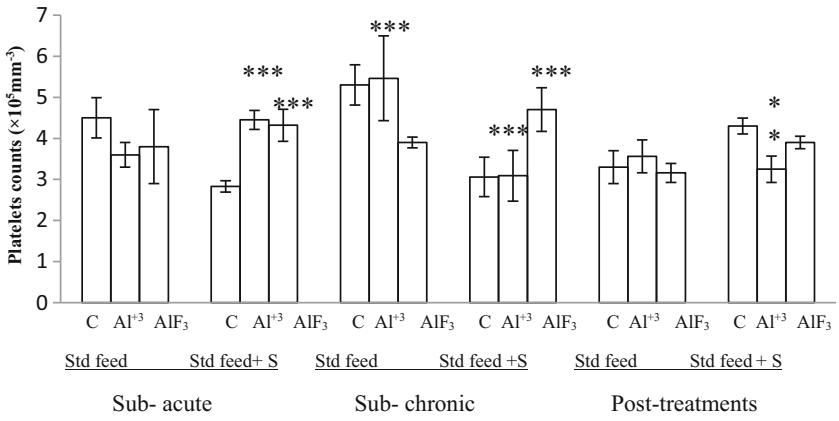

Fig. 9 Platelets counts of controls, Al^{+3}, and AlF_3-treated mice (Sub-acute, Sub-chronic, and Post-treatments) receiving different feeding groups *Std* standard feed, *S* Spirulina. Significant at $**p < 0.01$, $***p < 0.0001$

In contrast to RBC, WBC counts increased (54–124 %) in the treatments of standard feed groups, particularly of sub-chronic (Fig. 8). This has been ascribed to activation of the immune system in response to toxic action of test chemicals; a normal cell mediated immune response (El-Demerdash 2004; Abdel and Zabut 2011; Kumari and Kumar 2011). The increase in WBC counts in Spirulina groups was, however, poor possibly because of suppression of toxicity by antioxidants. WBC counts decreased in the post-treated mice in comparison to post- controls possibly on account of reduction in concentration of Al^{+3} in the serum that normalized hemopoietic system (Milovanovic et al. 2012).

Test chemicals altered differential leucocyte counts. Compared with controls, percentage of neutrophils and monocytes increased in the treatments while that of lymphocytes decreased (Tables 2 and 3). The percentage of eosinophil and basophil, however, differed little with the controls. The alterations in percentage of differential leucocyte counts were higher in standard feed groups compared to Spirulina groups. Further recovery of post-treated mice of Spirulina groups was also better (Table 4). The reduction in lymphocyte coupled with increase in neutrophil and monocyte percentages is possibly associated with pathology in treatments of standard feed groups (Sharma et al. 2013).

The platelet counts decreased (15–26 %) in standard feed treatments in comparison to controls. Supplementation of Spirulina increased platelet counts significantly compared to controls (53–56 %, Fig. 9). No such alteration was observed in platelet counts of post-treatments. Kaupke et al. (1993) reported increase in erythropoiesis and platelet production in dialysis patients supplemented with recombinant human erythropoietin. Phycocyanin pigment of Spirulina stimulated production of erythropoietin (EPO) hormone (Henrikson 1994) that possibly favored platelets production in Spirulina treatments.

Conclusion

In the present study, test chemicals were found more toxic during sub-acute exposure suggesting dose-dependent toxicity. Because of higher alterations in values of hematological parameters, we conclude AlF_3 to be more toxic than Al^{+3}. The richness of iron, vitamins, and antioxidants in Spirulina possibly reduced Al^{+3} and AlF_3 toxicity in mice. Since Spirulina is safe for human consumption (Hirahashi et al. 2002), it may therefore, be used as diet supplement in patients suffering from aluminum toxicity.

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