

Effect of *Arthrospira platensis* against sodium fluoride-induced haematological alterations

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The objective of present study was to investigate the effects of hydroalcoholic extract of *Arthrospira platensis* (ASP) against sodium fluoride (NaF) induced hematological alterations. Thirty-six male Wistar albino rats were divided into six groups of six animals each. Group I served as normal control. Group II served as toxic control. Group III served as plant control received ASP at a dose of 400 mg/kg body weight (*p.o.*). Groups IV-VI served as treatment groups, which received the hydro alcoholic extract of ASP at doses of 100, 200 and 400 mg per kg body weight (*p.o.*), respectively. All except group I and III received NaF (100 ppm) through drinking water for 30 days. Various blood parameters such as leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count were estimated. Results showed that ASP restored fluoride-induced hematological changes. In conclusion, the present study revealed that ASP has the good mitigative effect against sodium fluoride-induced hematological changes.

Keywords: Hematological profile, Sodium fluoride, *Spirulina*

Fluorine is one of the most abundant, widely distributed and predominant environmental pollutant in the world¹. Groundwater is one of the significant sources of fluoride, considered as the potential cause for several diseases. It is prevailing in 23 countries around the world including India, where it is found to be endemic at around 18 out of 33 states and union territories². The reason attributed is industrialization and lack of proper inexpensive techniques for purification³. Though fluoride is a significant element for the growth of bones and dental cavity prevention, its necessity is limited (<1 ppm) above which it leads to skeletal and non-skeletal fluorosis⁴.

Fluoride usually enters in the blood circulation by the passive diffusion process from the gastric and duodenal mucosa. Non-skeletal fluorosis is a clinical condition in which the soft tissues affected by inducing oxidative stress⁵. The toxic effects of fluoride on blood profile are well documented; it alters the anatomical and functional capacity of bone marrow by increasing the production of superoxide radicals and lipid peroxidation in polymorphonuclear

leucocytes⁶⁻⁹. Fluoride intoxication causes anemia and echinocyte formation through membrane degeneration¹⁰. Several studies reported that natural antioxidants dissuade fluoride-induced hematological changes^{11,12}. Agha *et al.*, 2012 observed that treatment with antioxidant vitamin E in combination with methionine and L-carnitine had effectively antagonized sodium fluoride-induced hematological alterations in experimental albino rats¹³.

Arthrospira platensis (also referred as *Spirulina platensis*) is commonly known as *Spirulina*. Its origin found in Aztecs of Mexico Valley and the Chad Lake of Africa in the 16th century. According to the available data, it is the first oxygenic photosynthetic, free-floating filamentous, spiral-shaped, non-heterocystous and multicellular form. *Spirulina platensis* appears as specific blue-green microalgae on earth since 3.6 billion years. According to the bacteriologist, it is prokaryotic due to its similar structure to that of bacteria, but according to botanists, it is classified as Cyanophyceae due to its chlorophylla¹⁴.

The *Arthrospira platensis* (ASP) is rich in nutritional and medicinal values. It contains Carbohydrates, proteins, dietary fiber, fats,

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water-soluble vitamins and minerals like calcium, iron, magnesium, phosphorus, iodine, and selenium along with Carotenoids. Scientific reports indicated that ASP has multiple pharmacological actions such as neuroprotective, hepatoprotective and nephroprotective¹⁵⁻¹⁷, anti-diabetes¹⁸, anti-obesity¹⁹, hypolipidemic, antioxidant and anti-inflammatory^{20,21}, cytoprotective²², antimicrobial effects²³ and also useful in the treatment of anemia and leukopenia²⁴ due to the presence of nutritional supplements and secondary metabolites. The present study aimed to investigate the effect of hydroalcoholic extract of ASP on the sodium fluoride-induced hematological alternations in Wistar male albino rats.

Materials and Methods

Collection and authentication of plant material

Arthrospira platensis freeze-dried powder purchased from Parry Nutraceutical, Division of EID Parry (India) Ltd., Chennai, Tamil Nadu state, India, and authentication done by Dr. Sunita Garg, Chief scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR; Voucher specimen was stored in the Department of Pharmacology, CMR College of Pharmacy, Hyderabad, Telangana state, India.

Preparation of the extract

Arthrospira platensis powder was extracted separately with various proportions of water and ethyl alcohol solvents (50:50, 30:70 and 70:30, respectively) and kept at 25°C for one week with occasional shaking. After that, it was stirred for 20 min and filtered. The filtrates dried in rotary evaporator (ROTA VAP) apparatus, and the suitable extract was selected based on the percentage of yield and stored in a refrigerator at 4°C for further studies.

Preliminary phytochemical screening

The Extract was subjected to initial phytochemical testing to determine the presence of secondary metabolites²⁵.

Experimental animals

Male Wistar albino rats (36 no.) weighing in between 220-250 g procured from Albino Research & Training Institute, Hyderabad, India. The animals acclimatized for ten days before starting the experiment. Rat feed was provided with water ad libitum and maintained a photoperiod of 12 h light/dark cycle. The study completed as per the guidelines of Committee for Control and Supervision

on Experiments on Animals, Government of India, after approval from the Institutional Animal Ethics Committee (IAEC No: CPCSEA/1657/IAEC/CMRCP/PhD-15/40).

Acute Toxicity studies

Acute toxicity studies were conducted to determine the maximum tolerable dose of extract according to OECD 425 guidelines. In this study, two groups of female Wistar rats (n=3 in each group) used. The first group has given with ASP 2000 mg/kg *p.o* and second was with the vehicle, distilled water. After administration, the animals were observed periodically for 30 min, during the first 24 h, with better attention for the first 4 h, daily. After that regularly noticed for a total of 14 days for the profiles such as lethargy, alertness, irritability, spontaneous activity, changes in skin, fur, eyes and behavior pattern, tremors, convulsions, salivation, defecation and urination, coma and death.

Experimental design

The dose of NaF was selected based on the previous study²⁶. After ten days of adaptation period, the experimental animals divided into six groups of six animals where the Group I served as the normal control, Group II as the toxic control. Group III served as plant control and supplied with 400 mg ASP per kg body weight (*p.o*). Groups IV, V and VI, served as treatment groups, which are given with hydroalcoholic extract of ASP at doses of 100 mg, 200 mg, and 400 mg per kg body weight (*p.o*), respectively. All groups except for group I and III are given with NaF (100 ppm) through drinking water for 30 days.

After the treatment schedule, animals fasted overnight, and blood was collected by puncturing the retro-orbital plexus. Blood samples were collected in K₂ EDTA tubes for estimation of various hematological parameters such as leukocyte count, erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and platelet count by using Medonic hematology cell counter.

Statistical analysis

The values expressed as Mean \pm SEM, n=6 in each group. The statistical analysis was carried out by one-way analysis of variance (ANOVA) followed by Post hoc Dunnett's test using Graph pad prism 5.0. The values were significant at $P < 0.05$.

Results

Selection of plant extract

The percentage yields of the various ratios of water and ethyl alcohol (50:50, 30:70 and 70:30) was found to be 14, 16 and 13%, respectively. Based on obtained percentage of yield, we selected 30:70 (Water: Ethyl alcohol) extract of *Arthrospira platensis* (ASP) for further studies.

Preliminary phytochemical investigation

The phytochemical screening of the selected hydroalcoholic extract of ASP showed the presence of alkaloids, glycosides, steroids, flavonoids, phenols, and tannins as major secondary phytoconstituents.

Acute toxicity study

Acute toxicity study of ASP showed well tolerance up to the dose of 2000 mg/kg b. wt (*p.o.*). All the animals in the treatment groups were normal in alertness and behavior up to 72 h of post administration. No mortality observed until completion of the study. Therefore 1/20, 1/10 and 1/5 of this test dose was selected as low (100 mg/kg), medium (200 mg/kg) and high (400 mg/kg) *p.o* doses for screening the alleviatory effects against sodium fluoride-induced toxicity.

Effect on the hematological profile

Hematology profile of the ASP treatment was presented as Mean \pm SEM in (Table 1). In Group II, WBC, MCHC, and Lymphocyte count increased whereas decreased levels of MCV, Platelet and serum iron ($P < 0.001$) observed when compared to the Group I. No significant variation observed in the levels of RBC, Haemoglobin, Haematocrit, and MCH in Group II. In ASP treatment, increased WBC count, decreased MCHC and lymphocyte counts, normalized MCV, and platelets count

observed in a dose-dependent manner (Group IV, V and VI).

Discussion

Blood is an important fluid connective tissue for oxygen and nutrient supply to the needs of the various tissues. Also, it can act as a relay station for transportation and also eliminate endogenous, exogenous waste inactive/toxic metabolites from the body. When the concentration of these metabolites increases, it could not only cause significant alternations in the composition and function of the haemopoietic system but also induces or precipitate the damage of other systems.

Fluoride is one of the prominent and conspicuous environmental pollutants. Kamble and Velhal 2010; Choudhary *et al.*, 2012; Sharma *et al.*, 2013 reported that fluoride could produce dose and duration dependent pathological alterations in complete blood picture²⁷⁻²⁹. In the present study, fluoride-exposed rats showed the significant increase of leukocyte count in the fluoride control group and possible mechanism involved in the increased leukocyte count by the stimulation of immune system due to the increased production of free radicals by oxidative damage of fluoride. The present result was correlating with the above reports and treatment with ASP at 100, 200 and 400 mg/kg b.wt. doses showed a significant increase in leukocyte count might be due to its immune boosting property³⁰.

Fluorine is a highly reactive electronegative element in the halogen group, and its negative charge attracted by positively charged ions like aluminum, calcium, and sodium. Tooth and Bones have the highest amount of calcium in the body, and they cause the maximum amount of fluorine which to be accumulated as calcium fluoro-apatite and finally unbound calcium excreted

Table 1 — Effect of ASP treatment on blood picture against NaF induced toxicity

Name of the parameter	WBC ($\times 10^3$ cells/ μ L)	RBC ($\times 10^6$ cells/ μ L)	HGB (g %)	HCT (Vol %)	MCV (Cubic micron)	MCH (Pictogram)	MCHC (%)	PLT (Lakhs/Cumm)	LMY (%)	Serum Iron (μ g/dL)
Group I	7.89 \pm 0.35	8.15 \pm 0.54	14.58 \pm 0.65	49.82 \pm 3.64	63.68 \pm 3.12	18.10 \pm 0.85	28.47 \pm 0.49	757.7 \pm 60.32	68.37 \pm 3.19	109.6 \pm 3.12
Group II	12.22 \pm 1.2	8.48 \pm 0.09	15.65 \pm 0.27	45.07 \pm 0.75	54.33 \pm 1.23 ^f	18.46 \pm 0.70	34.67 \pm 0.45 ^d	573.3 \pm 6.67 ^a	78.17 \pm 2.18	41.75 \pm 8.68 ^d
Group III	14.10 \pm 0.80 ^f	8.52 \pm 0.14	15.83 \pm 0.40	53.97 \pm 0.90	63.30 \pm 0.03	18.64 \pm 0.75	29.54 \pm 1.17	728.33 \pm 19.32	73.67 \pm 0.69	245.69 \pm 6.36 ^{ad}
Group IV	11.95 \pm 0.24	8.55 \pm 0.14	15.05 \pm 0.53	44.35 \pm 0.95	51.93 \pm 0.26	17.62 \pm 0.91	33.95 \pm 1.91	592.5 \pm 8.13	78.55 \pm 2.37	57.70 \pm 1.21
Group V	12.34 \pm 0.51	7.90 \pm 0.11	14.52 \pm 0.21	42.42 \pm 0.67	53.13 \pm 0.30 ^f	20.25 \pm 1.04	33.83 \pm 0.09	759.20 \pm 14.54	77.00 \pm 1.25	153.24 \pm 8.02 ^a
Group VI	13.42 \pm 1.18	8.04 \pm 0.18	16.00 \pm 0.23	44.70 \pm 0.99	55.29 \pm 1.28	26.64 \pm 1.34 ^{ae}	35.54 \pm 0.73	800.00 \pm 19.29	80.00 \pm 1.42	208.29 \pm 3.85 ^{ad}

Values are represented as Mean \pm SEM. Statistical analysis performed using one way ANOVA followed by post hoc Dunnett's multiple comparison test. $P < 0.001$ and $^bP < 0.01$ Vs Group II; $^dP < 0.001$, $^eP < 0.01$ and $^fP < 0.005$ vs Group I.

from the tissue. Several studies reported that excessive intake of fluoride hampers haemopoiesis, alters blood parameters and absorption, protein binding, distribution and excretion of minerals. The biochemical changes in the glucose metabolism in erythrocytes have been related to the structural and functional alterations of red blood cells during erythropoiesis by accumulated fluoride in red bone marrow³¹. Some research studies reported that fluoride caused significant changes in blood parameters such as RBC, Hb, Hct and MCH^{9,27} but in the present study, no statistical differences observed in these parameters after treatment with ASP with doses of 100, 200 and 400 mg/kg b.wt. and also in ASP control groups.

Fluoride can have not only a direct effect on water-soluble vitamins function but can have an indirect effect by its antiseptic nature thus altering the normal microbial flora in the intestine and also reduce the production of hydroalcoholic acid in the stomach and intrinsic action. This leads to decreased folic acid and vitamin B₁₂ levels in the body which are essential for many important metabolic processes such as synthesis and repair of DNA. Also, it has been proven associated with anemia, mental retardation, low IQ, neuronal tube defects, spinal cord malformations, dysplasias and nervous system abnormalities^{6,32,33}. In the present study, elevated MCHC level may indicate the folic acid deficiency in the toxic control group when compared to the normal group. No significant variation was observed in ASP treatment and ASP control groups when compared to the normal group.

Lowered MCV is one of the indications of iron deficiency anemia³⁴. Significantly decreased level of serum iron was observed in the toxic control group when compared to the normal group. Administration of hydroalcoholic extract of ASP leaves showed increased serum level of iron in a dose-dependent manner. The possible reason behind in the significantly increased serum levels of iron might be due to their relative richness of iron and vitamin contents³⁵.

Decreased Platelet count observed in the present study in toxic control was probably due to the harmful effects of fluoride on bone marrow and hematopoietic organs when compared to the normal group^{36,12}. Administration of ASP at doses of 100, 200 and 400 mg/kg b.wt. showed increased serum level of platelet count in a dose-dependent manner which reflects protective nature of ASP against fluoride-induced bone marrow suppression.

Conclusion

Treatment with *Arthrospira platensis* showed a significant increase in the leukocyte count indicates its potential immune stimulatory effect. Increased serum iron levels observed in a dose-dependent manner after treatment with *Arthrospira platensis* which might be due to the presence of high iron and vitamins content. No statistical difference observed in RBC, Hb, Hct and MCH levels.

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