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Study of anticlastogenic effect of spirulina against arsenic using zebra fish by micronucleus method

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Abstract

Arsenic is a potent toxic chemical that causes ground water contaminant. Nowadays different health hazards causes due to Arsenic toxicity when common people exposed to contaminated water. To reduce harmful toxic effect of Arsenic it is a big challenge for researcher to find out the easy and cheaper way. The study aims to explore the potential of using Spirulina which can ameliorate the toxic effect of Arsenic by its antagonistic properties in zebra fish. We use zebra fish as a model organism which uses as indicator for aquatic pollution study. This experiment 10 ml stock solution sodium arsenite (30mg dissolved in 100ml) is used. The present study shows that Spirulina a known antioxidant food, already used by human being is able to decrease is able to decrease moderately the incidence of micronucleus formation.

Key- words: Arsenic, Spirulina, Micronucleus

Introduction

The environment is getting to be more and more contaminated with heavy metals due to interference of human activities like mining's, from manufacturing companies, waste disposal and fuel combustion etc.Anthropogenic disturbance causes a serious threat to aquatic ecosystem for dissolving with waste materials and ultimately heavy metals. High level of toxicity like chromium, lead, mercury, arsenic and cadmium are the heavy metals that are accumulated in water and sediments causes' pressure to the food chain and also causes acute and chronic diseases in human. Heavy metals change the metabolic process; make DNA damage and may possible to carcinogenesis or apoptosis. Fish is considered to be a good bio-indicator which helps to accessing the potential danger of chemical introducing in aquatic ecosystem. Among the the technique Micronucleus test is more relevant test for fish. This test is applicable for screening the genotoxic compound.

The micronucleus test for fish has proved to be a useful technique for accessing the genotoxic properties of compound present in aquatic environment.(AL-Sabtiand Metcafe1995). The micronuclei [MNS] form by the loss of acentric fragments or whole chromosomes that are not incorporated in the brother nucleus during mitosis, by apoptosis, or phagocytises, among other mechanisms (Heddle JA, Cimino MC, Hayashi M, Romagna F, Shelby MD, Tucker JD, Vanparys P, MacGregor JT 1991).Basu et al. investigate individuals exposed to arsenic through drinking ground water (containing 368.11 mg/l of As) in West Bengal, India, and found a statistically significant increase in micronucleus frequency in oral mucosa cells, urothelial cells and lymphocytes (5.15, 5.74 and 6.39/1000 cells, respectively) when compared with unexposed controls (0.77 , 0.56 and 0.53/1000 cells, respectively). Fish study may provide important scientific data about toxicity on human beings. The study is to observe arsenic on fish and of using Spirulina which can ameliorate the toxic effect of Arsenic by its antagonistic properties in zebra fish. We use zebra fish as a model organism which uses as indicator for aquatic pollution study.

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Red mark denotes arsenic prone district in W.B

Material and Methods

We use zebra fish 3 months old with an average weight of 0.3 g were obtained from a pet shop. This fish is maintained in aquarium with oxygen pumps, thermometer and gives earthworms for feeding. Siphoning is done every day because at the bottom of aquarium dead eggs and uneaten food makes the water hazy. 10 ml stock solution sodium arsenite (30mg dissolved in 100ml) is used. We kept the fishes in each of the two beakers (control and experimental) for 120 hours. At first fishes will taken out of the water and blood was collected from gills. Then 15 slides were taken, saline solution was given on each slide. 1 drop of collected zebra fish blood was given on the slides from the collected gill of the fish through micropipette and slides will smeared. Smear was made from the bloods drops with cover slips. And then dried on the hot plate for 2mins.Slides were stained with freshly prepared 10% giemsa stain for 25mins.Stained slides were washed in normal tap water until the extra stain got washed out and apply tissue paper on the opposite side. Washed slides were left for being air dried.

Treatment of *Danio rerio* to Sodium Arsenite ($NaAsO_2$)

We maintained control (1lt 200ml water) and experimental as described above. 1 litre and 190 ml water + 10 ml stock solution of Sodium Arsenite (30 mg dissolved in 100ml) kept it for 120 hours. After 120 hours we follow the above steps.

Treatment of *Danio rerio* to Spirulina

We maintained control (1lt 200ml water + Spirulina) and experimental as described above. 1 litre and 190 ml water + 10 ml stock solution of Sodium Arsenite(30 mg dissolved in 100ml)+ Spirulina kept it for 120 hours. After 120 hours we follow the same steps.

Finally micronucleus was observed from processed blood taken from the treated fish.

Results and Discussion

Micronucleus Frequency

From the experiment we observed, when treated with only sodium arsenite, total number of normal cells 180, micronucleus 59 and abnormalities 32.77%. Again when treated with only spirulina the total number of normal cells 185, micronucleus 16 and abnormalities 8.64%. Result of Control gives total number of normal cells 170, micronucleus 5 and abnormalities 2.94%.

Micronucleus frequency always increases with time in fish in relation to genotoxicity, which was greater when treated with as than in fish treated with spirulina. When treated with both (sodium arsenate + Spiraling) the cells turn over sub lethal condition decreases micronucleus frequency, total number of normal cells 171, micronucleus 27 and abnormalities 15.78%. The micronucleus observations are showed on Figure 1.The final results showed in the Table 1 and Figure 2(a,b,c).

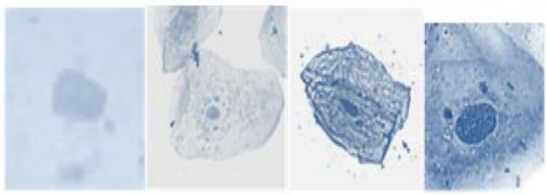

Fig. 1: Micronucleus

Table 1: Results of the study

Sl. No	Chemical Name	Total no. of normal cells	Micronucleus cells	% of abnormality
1.	Only spirulina	185	16	8.64%
2.	Only Sodium Arsenite	180	59	32.77%
3.	Sodium Arsenite+Spirulina	171	27	15.78%
4.	Control	170	5	2.94%

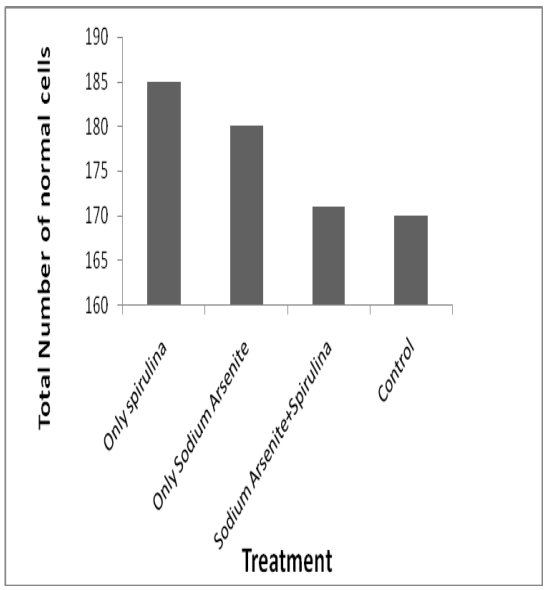

Fig. 2(a): Bar diagram represent comparisons between the expose treatment and controls shows the highest number of total normal cells

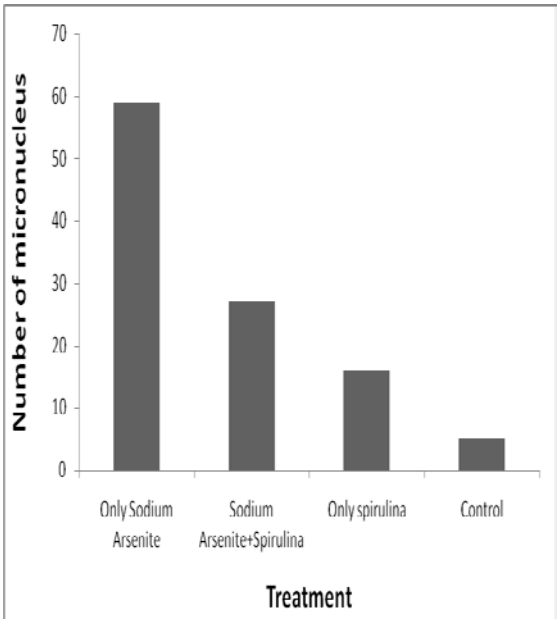

Fig. 2(b): Bar diagram represent comparisons between the expose treatment and controls shows the highest number of micronucleus

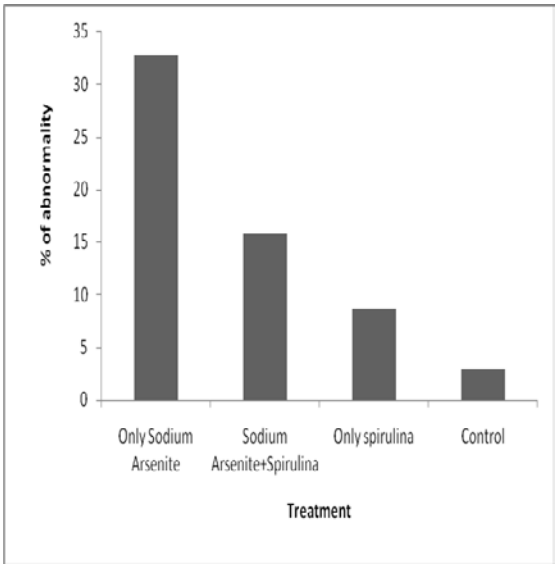

Fig. 2(c): Bar diagram represent comparisons between the expose treatment and controls shows the highest percentage of abnormalities

Conclusion

Toxicity screening on this model organism may provide important scientific data about arsenic toxicity on human being also. The present study showed that Spirulina a known antioxidant food, already used by human being is able to decrease moderately the incidence of micronucleus formation.

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