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Research Article

CONFINEMENT OF MICRONUCLEUS ON *DAWKINSIA TAMBRAPARNIEI* BY THE PARADOXICAL EFFECT OF THE ARSENIC DERIVATIVES

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ABSTRACT

Objective: In the current study, *in vivo* genotoxic effects of arsenic derivatives such as arsenic trioxide (As_2O_3) and sodium arsenite ($NaAsO_2$) on peripheral blood erythrocytes of *Dawkinsia tambraparniei* were investigated using the micronucleus (MN) test.

Methods: MN staining was done using acridine orange pre-coated slides. Fluorescent microscope was used for scoring.

Results: In $NaAsO_2$ exposed, the erythrocytes highest value was recorded at 42 days which is twofold higher than exposure at 7 days when compared to As_2O_3. Highest percentage was recorded about 13.9 in $NaAsO_2$, and in case of As_2O_3, it was recorded as 0.2% less. It was clearly confirmed that either form of arsenic is toxic to organism.

Conclusion: Anthropogenic activities have also brought in substantial amounts of them into the environment by mobilization from their natural insoluble deposits or environmental sins. Hence, arsenic pollution should have measured, and arsenic removal process should have carried out.

Keywords: Micronucleus, Blood, Arsenic trioxide, Sodium arsenite, Toxicity.

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INTRODUCTION

Current awareness of the potential hazards of heavy metals in the aquatic environment has stimulated much interest in the use of fishes as indicators of biomonitoring of environmental carcinogens, teratogens, and mutagens. Arsenic one of the major natural as well as natural pollutants produces biochemical changes in the organs of accumulated animals due to its possible toxicity. Through gills and skin, the arsenic compounds pass through it and produce toxicity. In groundwater, arsenic content has a moderate level of 1-2 µg/L, but in certain cases, the level was increased up to 1000 µg/Lin several countries including India [1]. The arsenic content in Thamirabarani river drinking water system, groundwater is slightly higher >0.01 mg/L, it is far recommended to curtail the used arsenic-bearing insecticides for agricultural activities and usage of arsenic filters on the sites of pumping stations of Thamirabarani river consuming water machine and at effluents locations of automobile and commercial areas at the upstream location of the Thamirabarani river [2].

The DNA molecule is a target site of most, if not all, carcinogenic and mutagenic agents. The micronucleus (MN) test is considered to be one of the most useful methods for evaluating genotoxicity in aquatic systems. MN is formed by chromosome fragments or whole chromosomes that lag at cell division due to the lack of centromere, damage, or a defect in cytokinesis [3]. Erythrocytes are the most commonly used cells in the piscine MN test. In fish, erythrocyte MN and nuclear abnormalities (NAs) also emerge unconsciously, and their frequencies may be reliant to the season [4].

Dawkinsia tambraparniei (Silas, 1953) is an endangered cyprinid found only in streams of the Thamirabarani river system in the South Western Ghats, India. As with many other stream fishes of India, there may be little or no records on the distribution [5]. In fish, exposure to chemical pollutants can induce either will increase or decrease in hematological stages. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners, and diseases [6]. Although arsenic toxicity greatly depends on its chemical forms, a few studies have taken into consideration the paradoxical phenomenon which is manifested by that sodium arsenite ($NaAsO_2$) acts as a mighty carcinogen; however, arsenic trioxide (As_2O_3) serves as a powerful therapeutic agent. In the present study, *in vivo* genotoxic effects of As_2O_3 and $NaAsO_2$ on peripheral blood erythrocytes of *D. tambraparniei* were investigated using the MN test.

METHODS

Test species

D. tambraparniei adults (length: 6±0.2; weight: 4±0.3) total of 100 fishes were caught by means of the fishing net at Papanasam (Thamirabarani river), Tamil Nadu, India. It was acclimatized in laboratory conditions in glass tanks for a minimal period of 14 days. It was maintained under temperature conditions of 25±2°C, 12 h/12 h light/dark cycle. At the time of the experiment, the fish was fed with *Artemia*.

Test chemicals

$NaAsO_2$ and acridine orange were purchased from Hi Media, Mumbai, and As_2O_3 was purchased from Sigma-Aldrich, UK.

Lethality study

The LC_{50} values for As_2O_3 and $NaAsO_2$ were determined using the arithmetic method of Sprague [7] and were set up to be 570 and 585 µM, respectively. Fishes were maintained in a glass tank of 25L capacity besides continuous air supply was given throughout the experiment. The physicochemical characteristics of test water were maintained accordingly by APHA standards, such as pH (7-7.2), total alkalinity (125-131 mEq/L), total hardness (200-205 mg/L as $CaCO_3$), calcium (50-56 mg/L), magnesium (18-22 mg/L), and DO (89-90 mg/L). At experimental duration, the water was changed every day and the fecal materials were taken away. All over the experiment, physicochemical parameters were set.

Experimental study

The observational work was conducted to examine the effect of As_2O_3 and $NaAsO_2$ by the method of Huntsman [8]. The fish was acclimatized

and grouped as follows in triplicate containing 7 fishes in each group and it was divided into Group I-III such as Group I: Negative control group, Group II: As_2O_3 intoxicated group, and Group III: $NaAsO_2$ intoxicated group. The fishes were exposed for 45 days (chronic).

MN analysis

After the experimental period was over, the blood of the exposed fishes was collected by a caudal puncture and coated with pre-cleaned slides. The slides were then fixed with ethanol for 20min and air dried. The smears were stained with acridine orange in a concentration of 0.003% dissolved in phosphate buffer. The stained slides were viewed under a fluorescent microscope and evaluated for the presence of MN exhibiting under a bright field in the blood. Short, non-refractive, round, or oblong chromatin bodies, demonstrating the same staining and focusing arrangement as the main nucleus, were scored as MN. The stained erythrocytic cells were viewed under an epifluorescent microscope with a magnification capacity of ×100 and appraise the presence of MN exhibiting bright orange colored. Erythrocytes were scored on each 1000 erythrocyte sample per fish. Obtained results were presented as a frequency (%). It was calculated using the formula:

$$\frac{1xMN + 2xBN + 3xTN + 4xTeN}{1000}$$

Where MN: Mononuleated, BN: Binucleated, TN: Trinucleated, TeN: Nucleated.

Statistical analysis

Means and standard errors were calculated for all data points from replicates. The means were compared between samples by Student's t-test analysis using SPSS, and $p \leq 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Each $NaAsO_2$ and As_2O_3 is the two foremost modes of inorganic trivalent arsenic. Importantly, $NaAsO_2$ is a properly documented carcinogen, even as As_2O_3 seems to be not only a poison, however, additionally an effective therapeutic tool in the treatment of acute promyelocytic leukemia and some stable tumors. To expose the difference among $NaAsO_2$ and As_2O_3 in the induction of genotoxicity in peripheral blood erythrocytes, MN assay was performed. On exposure, it was found that As_2O_3 possesses a more MN frequency when compared to $NaAsO_2$. These findings imply that $NaAsO_2$ and As_2O_3 differently have an effect on chromosomal breakage and DNA injury, which may be interlinked with their unique capacity to trigger oxidative stress [9].

As by days, the MN frequency got increased in As_2O_3 when compared to $NaAsO_2$. The changes in nucleus and MN formation are shown in Fig. 1. Thus, the exposure of arsenic-based MN frequency is purely on dose dependent, since a low dose of arsenic may also induce MN formation.

Susceptibility of arsenic in fish was determined to induce MN of their erythrocytes. Momentous increases in micronucleated cells have been determined in all the exposed fishes. The frequency of MN induction extended with corresponding growth in the exposure level, suggesting a dose-dependent increase in genotoxic indices. The MN assay needs a vast fraction of the cellular population treated with a genotoxic agent must undergo mitosis in order that the chromosomal anomalies prompted during the first mobile cycle are seen as MN within the cytoplasm at some points of the second or next cell cycles [10]. The frequency of cells nuclear bud and cells with MN improved considerably in fish exposed to the highest concentration. Lobed nuclei and blebbed nuclei were also observed in some cases [11].

The MN frequencies obtained among $NaAsO_2$ and As_2O_3 inductions were tabulated in Tables 1 and 2, respectively. Just like MN frequencies of $NaAsO_2$, the MN frequencies in fish exposed to As_2O_3 were notably higher ($p<0.05$). However, when compared As_2O_3 found to produce more MN frequency when compared to $NaAsO_2$. Comparison of MN frequencies in fish exposed to As_2O_3 and $NaAsO_2$ revealed a strong correlation between the expressions of MN and nuclear buds in peripheral erythrocytes. Interestingly, it was found that there is a slight decrease in MN frequency at 42 days of exposure. Since the fish was treated daily dealt with toxicant, there may be questionable remark that the fish will get adopted to the chemical for survival. However, the ratio of MN was not reduced drastically. The percentage of MN frequency in blood cells of *D. tambraparniei* after exposure to $NaAsO_2$ and As_2O_3 of 7, 14, 28, 35, and 42 days, respectively, was graphically represented in Fig. 2. The formation of MN and other nuclear dissimilarities may be due to caspase-activated DNase, resulting in cleavage of cytoskeleton

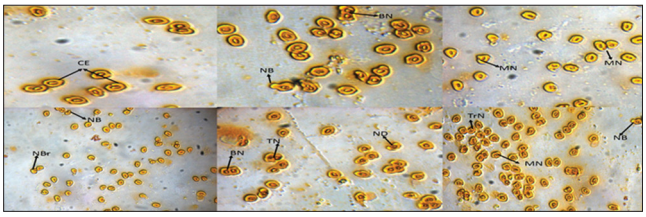

Fig. 1: Erythrocyte of fish stained with acridine orange and viewed under a fluorescent microscope. CE: Control erythrocytes, NB: Nuclear bud, NBr: Nuclear bridge, MN: Micronucleus, BN: Binucleated, TN: Trinucleated, TrN: Tetranucleated

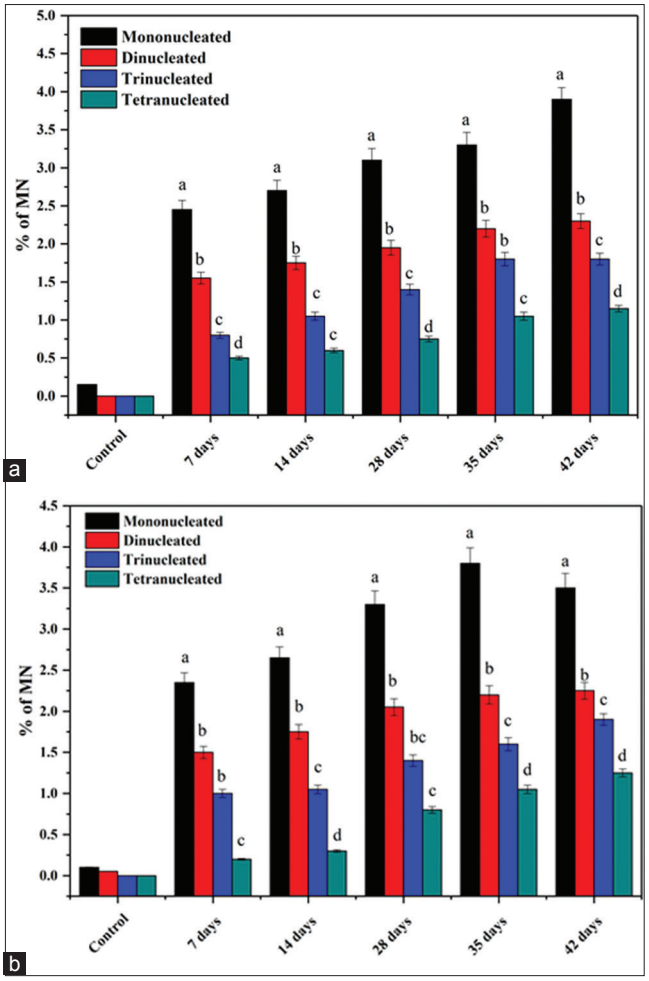

Fig. 2: % Micronucleus values in blood cells of *Dawkinsia tambraparniei* after exposure to (a) arsenic trioxide and (b) sodium arsenite. a, b, c, and d: Significant differences from the control at $p<0.05$ on exposure of 7, 14, 28, 35, and 42 days shown in column, respectively

Table 1: MN frequencies in erythrocyte of *D. tambraparnie*i exposed to sodium arsenite

Duration of exposure (days)	Number of cells scored	Number of cells with MN				Total	MN frequency (%)	Mean (%)±SE
		1	2	3	4			
Control	1000	1	0	0	0	1	0.2	0.03±0.047
7	1000	17	13	7	2	39	8.35	1.26±0.9
14	1000	21	16	9	5	51	9.3	1.43±1.002
28	1000	26	16	9	8	59	11.6	1.88±1.07
35	1000	32	22	12	9	75	13.004	2.16±1.18
42	1000	39	23	18	12	92	13.9	2.28±1.17

D. tambraparniei: Dawkinsia tambraparniei, MN: Micronucleus, SE: Standard error

Table 2: MN frequencies in erythrocyte of D. *tambraparniei* exposed to arsenic trioxide

Duration of exposure (days)	Number of cells scored	Number of cells with MN				Total	MN frequency (%)	Mean (%)±SE
		1	2	3	4			
Control	1000	2	0	0	0	2	0.15	0.03±0.075
7	1000	24	15	8	5	52	7.95	1.32±0.87
14	1000	27	17	10	6	60	9.35	1.52±0.91
28	1000	31	19	14	7	71	11.2	1.8±0.99
35	1000	33	22	18	10	83	13.1	2.08±0.93
42	1000	35	22	19	13	89	13.7	2.22±0.94

D. tambraparniei: Dawkinsia tambraparniei, MN: Micronucleus, SE: Standard error

(fodrin, vimentin, and gelsolin) and nuclear proteins, leading to mitochondrial damage [12].

In $NaAsO_2$ exposed, the erythrocytes highest value was recorded at 35 days. A number of MN counted for 1000 cells are about 32 which is twofold higher than exposure at 7 days. This indicates whenever there is a flow of continuous anthropogenic activities will be a chance of exhibition of more MN in affected aquatic species. A lower proportion of young erythrocytes in peripheral blood of *D. tambraparniei* was found to be 8.3%, and a higher proportion was found to be 13%. Likewise, $NaAsO_2$ in As_2O_3 exposed, the erythrocytes highest value was recorded at 35 days, and slight changes occurred in MN frequency at 42 days.

The outcomes of Ahmed [13] confirmed the importance of fish liver as capacity biomarker of arsenic toxicity for comet assay. The suitability of peripheral blood MN assay suggests its broader application as an early organic marker of exposure of fish to pollutants in the aquatic environment.

The genotoxic capability of acute concentrations of $NaAsO_2$ determined on this research advised severe difficulty toward its potential dangers to aquatic organisms. The average frequency of MN in fishes exposed to As (III) 12.03 was reported by Yadav [14] in *Channa punctatus,* but in this case the average frequency of 13.10 which is when compared the reported fish. Strunjak-Perovic [15] suggested that evaluation of erythrocytes in triploid rainbow trout did no longer reveal the presence of any morphological NAs in spite of substantially better MN frequency in relation to ordinary, diploid fish suggesting that correlation between NAs and MN is an end result of clastogenic but not aneugenic effects. Arsenic also causes damage to the liver as well as gills, in such cases, erythrocytes also got damaged [16,17]. Insufficient studies have taken into consideration to distinguish the precise molecular foundation of $NaAsO_2$ carcinogenicity and the anticancer effect of As_2O_3 in an experiment. In such case, the present study shows the affiliation between $As_2O_3$and $NaAsO_2$.

CONCLUSION

The present study conducted shows acridine orange staining that shows differentiation of NAs. In addition, with this paradoxical effect of arsenic compounds such as $NaAsO_2$ and As_2O_3 were seen. Arsenic in any form dissolves in water easily. Both chemicals have genotoxic effects in fish. The results of this study demonstrated that the river as well as stream fish species need to be conserved, hence, it is in endangered condition because the arsenic compounds cannot be destroyed easily in the environment, and thus *D. tambraparniei* test could be a sensitive and useful tool for evaluation of genotoxic effects in Thamirabarani river ecosystem.

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