Biospectra : ISSN: 0973-7057, Vol. 19(1), March, 2024, pp. 47-50

An International Biannual Refereed Journal of Life Sciences

Animal Science

ISSN : 0973-7057

Study on cyto- genotoxic potency of Arsenic trioxide on metaphase chromosome in Bone marrow cells of *Mus musculus*.

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Received : 12th December, 2023 ; Revised : 16th January, 2024

DOI:-https://doi.org/10.5281/zenodo.14166633

Abstract- The aim of the present study was to assess the cytotoxic and genotoxic potency of Arsenic on bone marrow cells of *Mus musculus*. For the research experiment, Adult *Mus musculus* of the same age group were selected for the research experiment and divided into three groups. After the completion of treatment, slides were prepared by the method of colchicine - hypotonic - aceto- alcohol - flame drying- Giemsa staining technique. For the assessment of mitotic chromosomal aberration 500 well- spread metaphase plates were screened randomly under the compound microscope at 100X (Oil immersion). The data obtained from the control and treated group are expressed as mean % ± SE and 't' test was used to determine by Excel Software and the level of significance is $p < 0.05$. The total chromosomal abnormality was 2.4 ± 0.68 (Control), 34.2 ± 2.12* (AA-1), and 58.8 ± 2.20** (AA-2) and it is a significant difference to control and treated groups at $p< 0.05$.

Key words: Arsenic, Bone marrow, Metaphase, *Mus musculus,* Chromosomal aberrations.

INTRODUCTION

Heavy metal contamination in biosphere has shown increase during past few decades.[1] Most heavy metals have the potential to induce mutation and are frequently referred as environmental mutagens, carcinogens and clastogens.[2] Arsenic is a major environmental mutagen and carcinogens: and reportedly induces various mutagenic, carcinogenic, and clastogenic effect in both plant and animals.[3] Arsenic has drawn the attention of scientific fraternity due to its toxic nature and widespread use in different commercial products. The genotoxic endpoints induced by Arsenic have been well-known for a long time. However, the genotoxic properties and mechanisms underlying the genotoxic effects of Arsenic are still unclear. It has been suggested that the mechanisms of the genotoxic effects of Arsenic could be involved with indirect mechanisms, such as the induction of oxidative stress contributing to DNA damage, the inhibition of DNA repair. The major mechanism of Arsenic toxicity is primarily involved in oxidative stress, described as an imbalance between the generation of reactive oxygen species (ROS) and the ability of antioxidants.[4] The genotoxic effect of heavy metals depends on its concentration and duration of exposure.[5] Hence, it is necessity to assess the cyto-genotoxicity induced by Arsenic.

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MATERIAL & METHOD

Experimental animals

For the research experiment, 15 adult Male Mice (*M. musculus*) of same age and average weight of 25 -30 gm

body weight were kept in a polypropylene cage under hygienic conditions in a well-ventilated room and were divided into equal number of mice in three groups one group was considered the control (C) group and the other two were considered treated (AA-1, & AA-2) groups.

Chromosomal Aberration Assay-

The mice were sacrificed by cervical dislocation 24h after administration of the last dose. Cytogenetic analysis was performed in bone marrow cells following the protocol of Preston *et al*. (1987)[6]. Experimental animals were injected (i.p.) with colchicine (2mg/kg) 1.5 h prior to sacrifice. Both femora were dissected out and cleaned of any adhering muscle. Bone-marrow cells were collected from both femora by flushing in KCL (0.075 M, at 37 °C) and incubated at 37 °C for 10 min. Collected cells were centrifuged at 1000 rpm for 10 min, and fixed in aceto-methanol (acetic acid: methanol, 1:3 v/v). Centrifugation and fixation were repeated three times at an interval of 10 min. The cells were resuspended in a small volume of fixative, dropped onto chilled slides, flame-dried and stained the following day with freshly prepared 4% Giemsa stain for 10-12 min, and washed in distilled water to remove excess stain. and 500 metaphase plate were examined under compound microscope with oil immersion lens.

STATISTICAL ANALYSIS

Data were analyzed using excel 2019 software. In each experimental variant obtained data from the control and treated groups are expressed as Mean % ± SE and for the comparison of data between the control and treated groups Unpaired 't'-test was used to determine at significant level $p < 0.05$.

RESULT & DISCUSSION

The metaphase analysis of bone marrow cells revealed various types of chromosomal aberrations, which consisted of chromatid and chromosomal types of gaps, breaks, unions, and fragments. Chromatid break, Acentric fragment, Ring, Chromatid gaps were noted to be more frequent than others. Relatively higher frequencies of Chromatid break were observed for all the doses of Arsenic. The results of the chromosomal aberration assay in bone marrow cells after oral administration with Arsenic are summarized in Table 1 and 2 and also shown in corresponding figure 1, 2 & 3. The frequency of chromosomal aberrations (CAs) also increased with increasing doses of Arsenic. It was a dose-dependent increase in genotoxic effect of Arsenic. Arsenic and its compounds are commonly regarded as a pollutant of worldwide concern which released by various industries. Arsenic is a cytotoxic that can increase the production of ROS (Reactive oxygen species) and can lower the antioxidant reserves in response to cell damage. The mechanism of Arsenic cytotoxicity increases the production of reactive oxygen species and lowers the reserve antioxidant. An increase of Reactive oxygen species will cause DNA damage and lipid peroxidation. Accumulation of ROS in germ cells especially superoxide radical (O_2^-) and Hydrogen peroxide radicals (H_2O_2) may Arsenic to apoptosis and cell death.[7]

Table 1: Incidence of chromosomal abnormalities in Bone marrow cells metaphase chromosome of *Mus musculus* with higher and lower dose of Arsenic Trioxide in 40 days duration.

Exp variant	Dose	Structural abnormality		Mitotic disruptive abnormality		Total abnormality	
		No.	Mean%±SE	No.	Mean%±SE	No.	Mean%±SE
Control	DW	8	1.6 ± 0.56	4	0.8 ± 0.39	12	2.4 ± 0.68
Lower Dose	0.04mg/kg. B. wt./per day	115	23 ± 1.88*	56	11.2 ± 1.41*	171	34.2±2.12**
Higher Dose	0.08mg/kg. B. wt./per day	199	39.8 ± 2.18*	95	19 ± 1.75*	294	58.8±2.20**

Table 2: Number of different types of abnormalities in bone marrow metaphase chromosome of *Mus musculus* with higher and lower dose of Arsenic trioxide in 40 days duration.

Exp Variant	Structural abnormality (SA)							Mitotic disruptive abnormality (MDA)					
	Ctb	Af	Ri	Ctg	Iso-Ctb	Iso-Ctg	Met	Poly	Clum	Stic	Pul	Hypo	C-mit
Control	2	0	2	2	1	1	0	0	0	1	1	1	1
AA-1	30	42	19	14	6	3	1	11	13	9	6	9	8
AA-2	59	57	38	22	11	7	5	18	21	15	12	18	11

Abbreviation

SA- Structural abnormality, MDA- Mitosis disruptive abnormality, TA- Total abnormality, Ctb- Chromatid break, Af- Acentric fragment, Ri-Ring, Ctg-Chromatid gap, Iso-Ctb - Iso Chromatid break, Iso Ctg- Iso Chromatid gap, Met- Metacentric, Poly- Polyploidy, Clum - Clumping, Pul- Pulverized, Hypo- Hypoploidy, C-mit- C-mitosis,

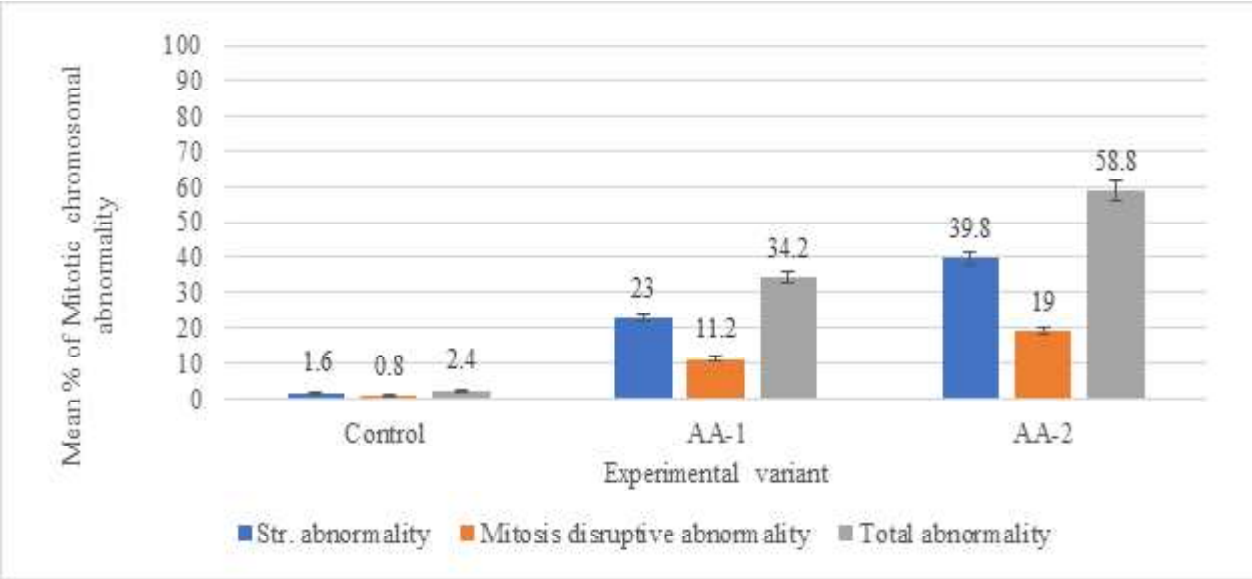

Figure 1: Histogram represents incidence of chromosomal abnormalities in mitotic metaphase of Bone marrow cells in 40 days of duration.

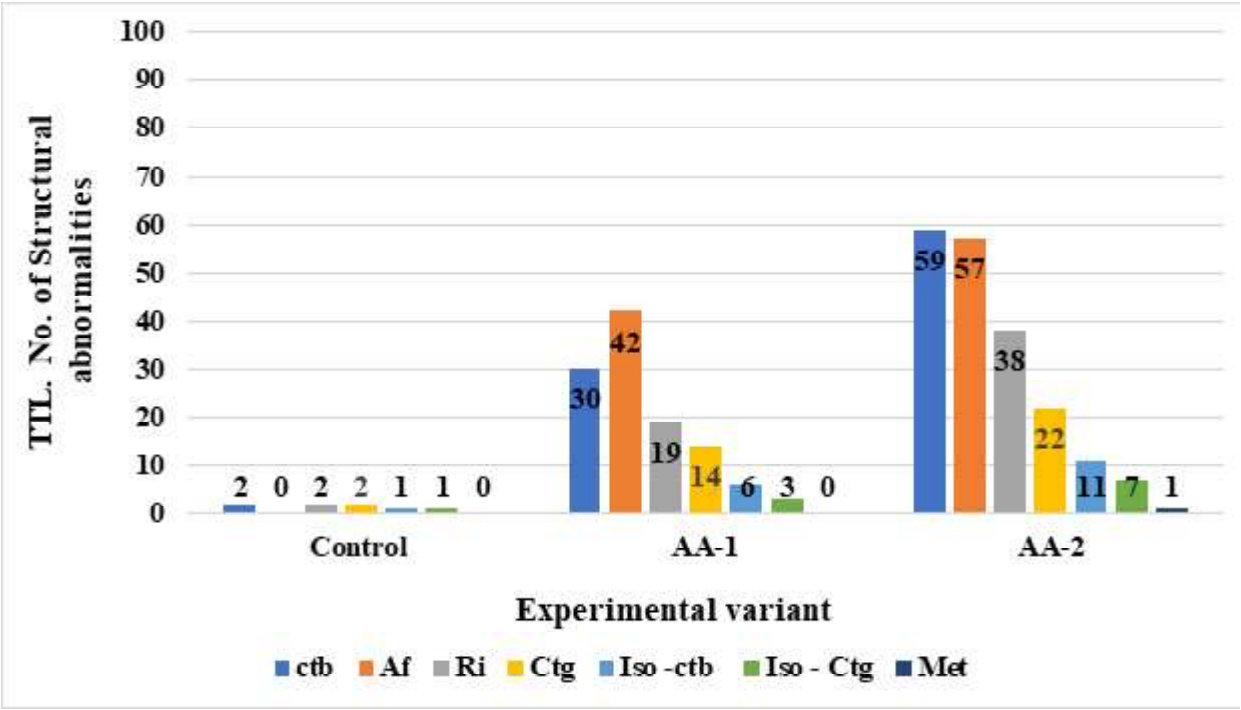

Figure 2: Histogram represents total number of structural abnormalities in mitotic metaphase of Bone marrow cells in 40 days of duration.

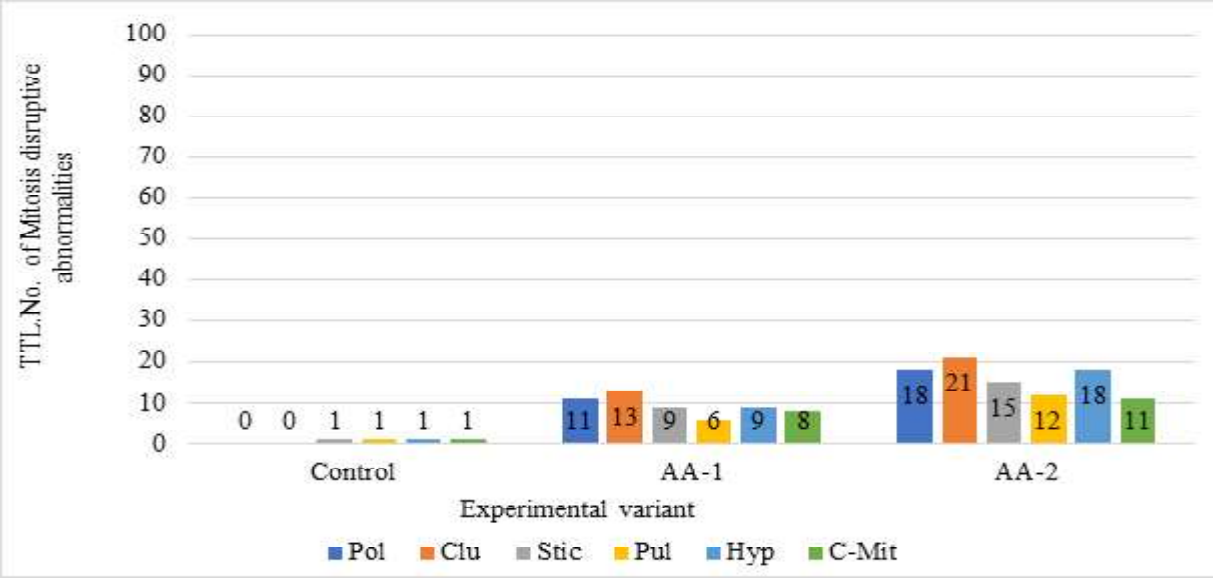

Fig 3: Histogram represents total number of Mitosis disruptive abnormalities in mitotic metaphase of Bone marrow cells in 40 days of duration.

CONCLUSION

From the obtained result data, it is concluded that Arsenic trioxide produces a cytogenotoxic effect on Bone marrow cells and increase the number of numbers of chromosomal abnormalities in metaphase chromosome of bone marrow cells in Swiss albino mice (*Mus musculus*).

ACKNOWLEDGEMENT

I would like to thank and express my heartfelt gratitude to Head Department of Zoology, B. N. Mandal. University, Madhepura, Bihar for his affectionate guidance and providing necessary laboratory facilities.

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