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Original Article

Assessment of Genotoxic Potential of Arsenic in Female Albino Rats at Permissible Dose Levels

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ABSTRACT

Background: Arsenic is a wide spread environmental contaminant and has been recognized as a genotoxic element which is of major public health concern. **Aim:** The present study evaluates the genotoxic potential of arsenic at low permissible dose levels. **Materials and Methods:** Forty-eight mature female rats were divided into four groups of 12 animals each. Group I animals received distilled water and served as control. Group II-IV animals received sodium arsenite dissolved in distilled water continuously for a period of 60 days at the dose of 10, 30 and 50 µg/L (ppb) respectively. Six rats from each group were sacrificed after 30 days of arsenic exposure and the remaining animals were sacrificed after 60 days. Liver was excised from the sacrificed animals to study the probable advent signs of carcinogenicity measured through microsomal degranulation test. Assessment of mutagenic potential of arsenic was evaluated through chromosomal aberrations observed in the bone marrow cells. **Results:** The levels of RNA and proteins decreased significantly ($P \leq 0.01$) in all the three doses administered along with an increase in % microsomal degranulation in hepatic fraction when compared to control at both 30 and 60 days time period. A dose-dependent increase in chromosome aberrations like fragmentation, breakage has been observed in all the treated animals. **Conclusion:** The results of present study revealed that chronic exposure of arsenic even at its low permissible dose limits results in carcinogenic and mutagenic effects which emphasize its genotoxic possibility.

Key words: Chromosome aberrations, genotoxic, microsomal degranulation, sodium arsenite

INTRODUCTION

The presence of arsenic in the environmental media results from both geogenic sources and anthropogenic activities. The occurrence of high concentrations of arsenic in ground water used for drinking purpose has been recognized as a major public-health concern in several parts of the world. Every day millions of people are exposed to arsenic via drinking water where the concentration of arsenic exceeds the permissible limit (10 µg/L) defined by the World Health Organisation (WHO).[1] Arsenic occurs in ground water in the form of arsenite, arsenate, methyl arsenic acid and dimethyl arsenic acid. Groundwater is predominately used for irrigation of agricultural crops which results in deposition of arsenic in crops and is the second largest contributor to arsenic uptake by people. Other potential sources of arsenic toxicity include the use of arsenic-contaminating herbicides, insecticides, rodenticides, preservatives and by products of fossil fuels.[2] Inhalation or ingestion of inorganic arsenic has been shown to cause cancer in humans, resulting in tumors of the skin, lung, liver, urinary bladder, and other locations, and has been classified as a proven human carcinogen by the International Agency for Research on Cancer (IARC),[3] in the EU (European Chemicals

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Bureau),[4] as well as by the US Environmental Protection Agency (EPA).[5] It has been reported that sub-chronic exposure to arsenic through drinking water alters the expression of cancer-related genes in liver,[6] increased the incidences of chromosome aberrations, sister chromatid exchanges and micronuclei in human populations.[7-9] Arsenic is not a direct DNA mutagen, but it diminishes DNA repair capacity and alters the DNA methylation patterns.[10] Existence of arsenic in different inorganic and organic forms, complicates its considerations on toxic effects. Toxicity varies according to its oxidation state, solubility and many other factors including the exposure dose, frequency, duration, species, age, gender, as well as individual susceptibilities, genetic and nutritional factors.[11-13] The well-known toxic effects of arsenic on human are difficult to reproduce in experimental animals,[14] but despite of this, the toxicological significance of low level oral exposure to arsenic and the dose response relationship for carcinogenic effects has been the subject of important discussion.

Although several *in vitro* studies have reported the genotoxic effects (carcinogenesis and mutagenesis) of arsenic at higher doses, the purpose of the present study is to focus on the evidence whether arsenic is capable of inducing/initiating genotoxic effects at low dose levels (10-50 µg/L) measured through hepatic microsomal degranulation and chromosomal aberration in bone marrow cells using female albino rats as an experimental model.

MATERIALS AND METHODS

Chemicals

Sodium arsenite and other chemicals used in the present study were purchased from S.D. Fine Chem. Ltd and were of analytical grade (AR).

Animals and experimental design

Forty-eight mature female rats were procured from Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, and acclimatized for 15 days before using them for experimentation. The rats were maintained under controlled condition of temperature (27 ± 2°C; 12h light/dark cycles) and provided with standard pellet diet and water ad libitum. The rats were divided randomly into 4 groups consisting of 12 animals each. Group I animals received distilled water and served as control. Group II, III and IV animals received arsenic as sodium meta arsenite at doses of 10, 30 and 50 µg/L(ppb) dissolved in distilled water for a period of 60 days. Half of the animals (6) from each group were sacrificed after 30 days of arsenic exposure and remaining others after 60 days.

Chromosome aberration assay

Experimental animals were injected (intraperitonealy) with colchicine (4 mg/kg) 1.5 h prior to sacrifice and cytogenetic analysis was performed on bone marrow cells.[15] 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 25 min. Collected cells were centrifuged at 3000 rpm for 10 min, and fixed in aceto-methanol (acetic acid:methanol, 1:3, v/v). Centrifugation and fixation were repeated five times at an interval of 20 min. The cells were resuspended in a small volume of fixative, dropped onto chilled slides, dried and stained the following day with freshly prepared 2% Giemsa stain for 3-5 minutes.

Microsomal degranulation assay

Liver (0.5 gram) was finely chopped and homogenized in 0.225 M sucrose tris (ST) buffer (pH 7.4) in chilled conditions and processed for microsomal degranulation.[16,17] Tissue homogenates were centrifuged for 20 min at 9000 rpm at 4°C, the post mitochondrial supernatant collected and mixed with 0.5 g calcium chloride. After that the tubes were kept in ice for 20 min, centrifuged at 4°C, 10,000 rpm for 20 min. The pelleted microsomes were resuspended in 0.225 M ST buffer (pH 7.4) and proteins, RNA were estimated as per the standard methods. Microsomal degranulation values above 5% were taken as positive result for representing carcinogenic properties of the chemical.[18]

Statistical analysis

Statistical analysis of the data for microsomal degranulation test was carried out by one-way analysis of variance (ANOVA). The values of treated rats were compared with control and the statistical differences were considered significant at $P \leq 0.05$, $P \leq 0.01$. All values were expressed as mean ± SEM.

RESULTS

Microsomal degranulation test

The observations recorded indicate that exposure of arsenic at low permissible dose limits is capable of inducing microsomal degranulation [Table 1]. The exposure to arsenic both for 30 and 60 days results in a significant decrease ($P \leq 0.01$) in RNA and proteins of treated rats when compared to control. Similarly, a dose-dependent increase in % degranulation has been observed in treated rats at both time periods of arsenic exposure. After 30 days of arsenic exposure, only 50 ppb (10.91%) and 30 ppb (7.43%) doses induced carcinogenic effects while 10 ppb dose caused only 4.51% degranulation which is considered as non-carcinogenic. However, all the three doses administered (50, 30 and 10 ppb) showed carcinogenic

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potential with per cent microsomal degranulation values of 14.04%, 7.60% and 5.82% respectively after 60 days of exposure [Table 1]. Hepatic fractions from the control group of rats showed 1.08% and 2.04% degranulation respectively for 30 and 60 days and were considered as non-carcinogenic.

Chromosomal aberrations

A significant increase ($P \leq 0.01$) in the chromosomal aberration in treated rats as compared to control animals indicating mutagenic behavior of arsenic [Table 2]. Structural chromosomal aberrations observed after exposure of arsenic were in the form of chromatid breakage (fragments, breaks and gaps). A dose-dependent increase in chromosomal aberrations was observed [Figure 1].

DISCUSSION

In the present study the carcinogenic potential of arsenic was assessed by measuring the detachment of ribosomes from rough endoplasmic reticulum (RER). Earlier studies have reported that carcinogens degranulated RER under *in vivo* and *in vitro* conditions[19] resulting in a decreased RNA/Protein ratio and provides the basis of a screening test for environmental or chemical carcinogens.[20] Liver provides a good model for the study of carcinogen-induced degranulation, mainly for two reasons: firstly, it was a rich source of rough endoplasmic reticulum and secondly, it has the metabolic capacity required to generate active forms of carcinogen from precursors. The administration of arsenic consecutively for 30 and 60 days in the present study resulted in a decreased RNA/Protein ratio[20] which has been taken as an index of degranulation. Our results are in consonance to the earlier findings[21] where a decrease in RNA/Protein ratio of treated rats due to direct membrane degranulation has been reported. Researchers have demonstrated that electrophiles of a carcinogen can disrupt ribosome membrane interaction in rough microsomes by their attack on nucleophilic components of the reticular membrane ribosome complex, involved in protein synthesis for export from cytosol.[22] Lack of exported proteins can adversely affect signal transduction across plasma membrane possibly leading to events at molecular levels leading to incidence of carcinogenesis.

Arsenic has been recorded as a genotoxic element and not a mutagen for both animals and humans.[3,7] The significant chromosomal aberrations observed in the present study were mainly in the form of chromatid breakages (gaps, break and fragments) which support the view of genotoxicity expressed earlier. Various *in vitro* studies revealed that arsenic can damage DNA and induces the formation of chromosome aberrations, micronuclei formation and sister chromatid exchange in mammalian cells.[14,23] Chromatid lesions occur only when chromosomes are damaged after G1 stage of the cell cycle leading to chromatid breakage.[24] Cytogenetic studies done earlier showed that arsenic exposure has a positive genotoxic effect and an increased number of chromosomal aberrations on human lymphocytes.[25,26]

Table 1: Effect of sodium arsenite on hepatic microsomal degranulation in female rats

	30 Days				60 Days			
	Control (Group I)	Arsenic-exposed group			Control (Group I)	Arsenic-exposed group		
		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)
Protein (mg/g) liver	4.46±0.04	4.23±0.05**	3.61±0.03**	3.21±0.02**	4.54±0.02	4.21±0.03**	3.50±0.02**	3.41±0.02**
RNA (mg/g) liver	3.86±0.21	3.52±0.16	2.92±0.059**	2.50±0.07**	3.88±0.19	3.47±0.13	2.83±0.07**	2.56±0.07**
RNA: Protein ratio	0.864±0.04	0.834±0.04	0.809±0.01	0.778±0.01	0.856±0.04	0.823±0.03	0.807±0.01	0.751±0.02*
Microsomal degranulation (%)	1.08	4.51	7.43	10.91	2.04	5.82	7.60	14.04

All values are mean±SE (n=6), **Values are significant at $P \leq 0.01$ level of significance. *Values are significant at $P \leq 0.05$ level of significance

Table 2: Assessment of chromosomal aberrations in the bone marrow cells of female rats after exposure to sodium arsenite

Chromosomal aberrations	30 Days				60 Days			
	Control (Group I)	Arsenic-exposed group			Control (Group I)	Arsenic-exposed group		
		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)		10 ppb (Group II)	30 ppb (Group III)	50 ppb (Group IV)
Fragments	0.25±0.25	0.62±0.23	1.5±0.24**	1.75±0.14**	0.5±0.28	1.75±0.14	2.75±0.47**	4.25±0.62**
Chromatid Breaks	-	-	0.25±0.25	0.5±0.28	0.25±0.25	0.37±0.23	0.62±0.23	1.25±0.25*
Chromatid Gap	0.12±0.12	0.25±0.20	0.50±0.28	0.75±0.25	0.18±0.16	0.37±0.23	0.62±0.12	0.87±0.31
End to end association	-	-	0.12±0.07	0.18±0.11	-	-	0.5±0.28	0.25±0.25

All values are mean±SE (n=4 in each group). *Values are significant at $P \leq 0.05$ level of significance. **Values are significant at $P \leq 0.01$ level of significance

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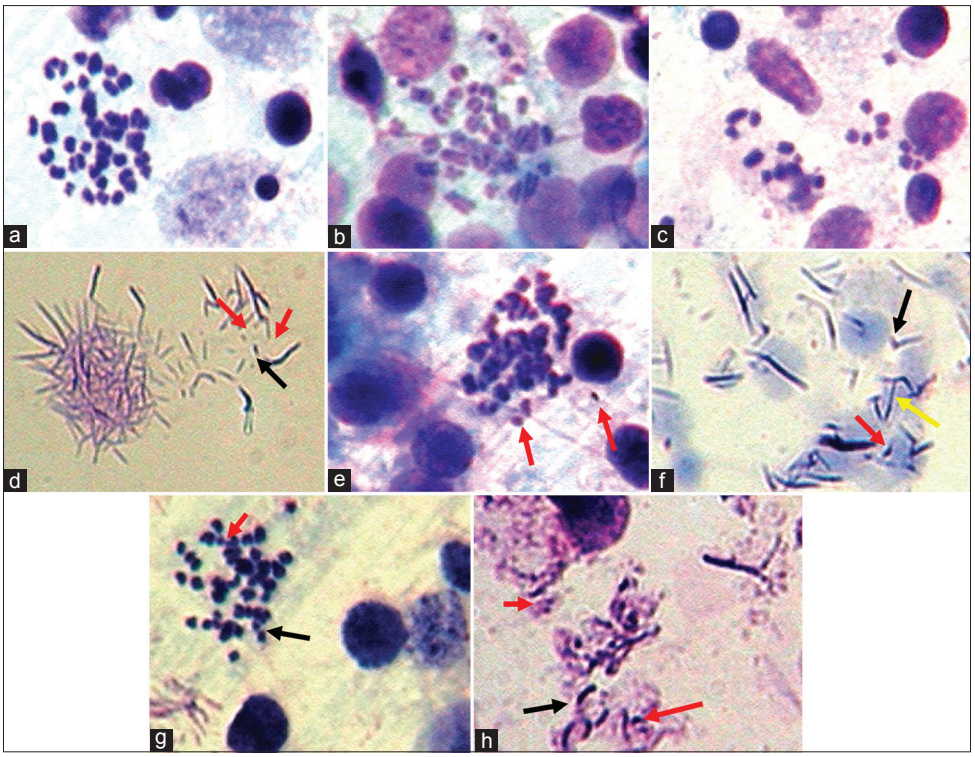


Figure 1: Photograph showing different types of chromosomal aberration. (a) Control rats, 30 days; (b) Control rats, 60 days showing normal metaphase plate. (c) 10 ppb, 30 days; (d) 10 ppb, 60 days showing fragmentation, chromatid gap and breakage. (e) 30 ppb, 30 days; (f) 30 ppb, 60 days show fragmentation, chromatid break and end to end association of chromosomes. (g) 50 ppb, 30 days; (h) 50 ppb, 60 days show fragmentation and chromatid breaks. Red arrow showing fragmentation, Red dotted arrow showing chromatid gap, Black arrows showing breakage and Yellow arrow showing end to end association

CONCLUSION

Chronic exposure of arsenic even at its low and permissible dose limits (10-50 µg/L) results in degranulation and chromosome aberrations which substantiates the possible genotoxic potential of arsenic in animals. However, further studies on animals are needed to hypothesize the detailed molecular mechanism involved in genotoxicity of arsenic laden compounds.

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