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Research Article

Contamination Effect of Arsenic Trioxide on White Rat

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Abstract
Widely present in the environment, arsenic trioxide has been identified as a genotoxic substance that poses a serious risk to public health. The genotoxic potential of arsenic at low allowable dosage levels is assessed in this study. Four groups of twelve adult rats each were created from the 48 total. Animals in Group I were used as controls Chromosome abnormalities found in bone marrow cells were used to assess the mutagenic potential of arsenic. Hematological parameters were also assessed. At 60 and 90 days, the percentage of microsomal degranulation in the hepatic fraction increased and the amounts of RNA and proteins considerably reduced ($P < 0.01$) in all three dosages given. was employed in order to assess hematological parameters White blood cells, lymphocytes, red blood cells, platelets, and mean erythrocyte hemoglobin concentration (MCHC) were all substantially lower ($P \leq 0.05$) in the arsenic treatment group than in the control group. Nevertheless, there are no appreciable changes in other measures such granulocytes, mean absolute count, hemoglobin (HGB), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), and platelet count (PCT). We found that when rabbits get repeated therapeutic dosages of ivermectin over a brief period of time, Therefore, it is advised to take one dose over a few weeks. All of the treated animals showed a dose-dependent increase in chromosomal abnormalities such as fragmentation and breaking. The current study's findings demonstrated that long-term exposure to arsenic, even at low allowable dosage levels, has mutagenic and carcinogenic consequences, underscoring the metal's potential for genotoxicity.

Keywords: Chromosome aberrations, genotoxic, microsomal degranulation, sodium arsenite

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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 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.[7,8,9] 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.[11,12,13] 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:

Animals and experimental design

Forty-eight mature female rats were procured from Department of biology in fuluja university Production and Management, 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 and dissection:

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] The results of our studies on the effect of therapeutic doses of ivermectin, administered repeatedly, on the hematological parameters of rabbits are presented.

The leukogram in this study indicated that leukopenia may be related to the toxic effects of ivermectin on cell.[27] (This Arsenic induces cell death through a mechanism related to its known chloride channel activator function) Tribiñoset al.(2023 ,.

Since arsenic is a lipophilic substance.[28] it interacts with other cells and affects their functions, which causes damage to organs, such as the liver)Arise&Malomo, 2009.(Thus, the result of our study is adecrease in the number of leukocytes in the treated group, this result is due to the body's response to the action of ivermectin, leading to an increase in leukocytes, and then a decrease due to its repeated doses.

On the other hand ,Abdel-Rahman&Ali .[29] found Arsenic - inducedleukocyosis in sheep in their study. Moreover, a decrease in the lymphocyte counts was observed as a result, which may be adverse effect of Arsenic on lymphocytes.

In the table (2), the results observed he effect of Arsenic on hematological values, it was reduced in RBC counts, MCHC and platelate counts, and no effect of another parameters involved) HGB, PCV, MCH and PCT.(This anemia may be caused by a shift in hematopoiesis caused by nephrotic injury, liver degeneration, or decreased bile salts in the small intestine. .[30] The lipophilicity of ivermectin also promotes rapid transport of the drug from the circulation to various tissues)Jourdan et al ,(,.thus interact with plasma proteins, especially proteins that transport minerals, in particular iron-binding proteins, so that they give a clear indication of how the number of erythrocytes, platelets and MCHC decrement are reduced in treated rabbits.

The observed changes confirm that frequent Arsenic dosages have a deleterious influence on rabbit health. Therefore, it is suggested to take a single dose each time.

The effects of Arsenic **on hematological parameters related with white blood cells) WBC(**

The results indicate a significant (P≤0.05)decrease in total white blood cell count in the treatment (Arsenic) group compared with the control group at both periods .As well as a decrease in lymphocytes, especially after 60 days. On the other hand, there is no difference in the mid-range of absolute numbers.

Table 1 :The effects of Arsenic **on hematological parameters related with white blood cells in rat.**

Parameters	Time	**Control**	**Ivermectin**	LSD
White blood cells /10^9)l(	**At 60 day**	0.32±12.1 Aa	0.3± 9.5 Ba	**1.43**
	At 90 day	0.15± 12.3 Aa	0.4± 8.5 Ba	
Lymphocyte/10^9)l(	**At 60 day**	1.1±5.1 Aa	0.4±5.6 Ba	**2.28**
	At 90 day	0.27± 4.6 Aa	0.26±1.78 Ba	
Granulocyte/10^9)l(	**At 60 day**	0.8±5.8 Aa	1.2±4.1 Aa	**3.25**
	At 60day	0.5± 4.3 Aa	0.5± 7.3 Aa	
Mid-range absolute count/10^9)l(	**At 90 day**	0.06± 1.46 Aa	0.12±0.89 Aa	**0.6**
	At 60day	0.05± 1.2 Aa	0.27±1.36 Aa	

Values are expressed as mean ± SE, each group containing five animals.

The individual capital letters indicate statistically significant differences between groups within the same line at (P≤0.05).The individual small letters indicate statistically significant differences between times within one column.

Conclusion:

Chronic exposure of arsenic even at its low and permissible dose limits (10-50 µg/L) results in degranulation and chromosome aberrations and hematological changes 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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