

RESTORATIVE EFFECTS OF *ZINGIBER OFFICINALE* ON ARSENIC-INDUCED GENOTOXIC EFFECTS IN CHROMOSOMAL STRUCTURE OF MICE

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ABSTRACT

Objective: The present study investigated the antigenotoxic effect of *Zingiber officinale* (Ginger) on arsenic-induced genotoxic effects in chromosome structure of Swiss albino mice.

Methods: Albino mice were divided into four groups: Group I was kept as control, Group II was administrated an oral dose of arsenic trioxide 5 mg/kg bw for 15 days, Group III was given an oral dose of arsenic trioxide 5 mg/kg bw followed by 20 mg/kg bw of ginger orally, and Group IV was administered with 20 mg/kg bw of ginger orally for 15 days and kept as positive control. The autopsies were done from all the groups on 15-day post-treatment, and bone marrow was removed for chromosomal study and for calculating the frequency of micronuclei (MN).

Results: It is concluded from this study that long-term arsenic exposure causes chromosomal anomalies, for example, breaks, gaps, rings, stretching, and stickiness which leads to the fragmentation of chromosomes and increases the frequency of MN.

Conclusion: The administration of ginger showed modulatory effects against arsenic-induced genotoxicity.

Keywords: Arsenic (As), Chromosomes, *Zingiber officinale*, Genotoxicity.

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INTRODUCTION

Arsenic contamination in groundwater has become a worldwide problem. Currently, an unprecedented number of people in West Bengal, India, and Bangladesh are exposed to the ubiquitous toxicant through drinking water to the levels far exceeding the maximum recommended limit laid down by the World Health Organization (WHO). Keeping in view the health hazards, arsenic is one of the most important environmental toxicants, and it has become evident that increasing human activities have modified the natural cycle of metals and metalloids [1-3]. The risk of arsenic compounds has been elevated due to increasing human activities such as mining, smelting, and pesticide production which has led to the accumulation of arsenic in the soil [4]. The trivalent arsenite is more toxic than pentavalent arsenate. The trivalent form has higher affinity for thiol group, and it is more cytotoxic and genotoxic [5].

Exposure to arsenic causes both chronic and acute toxicity and is also evaluated as a human carcinogen [6,7]. The chronic exposure to arsenic causes various types of carcinogenic and non-carcinogenic health effects such as cancer of skin and internal organs, diabetes mellitus, hypertension, and respiratory conditions [8,9].

Arsenic induces lipid peroxidation and thus acts as a prooxidant and also brings about protein and enzyme oxidation, glutathione depletion, DNA oxidation, and DNA adducts [10]. Arsenic leads to the formation of reactive oxygen species (ROS) like nitric oxide, which disturbs the poly ADP-ribosylation which is necessary for DNA repair, signal transduction, and apoptosis. As a result, arsenite induces DNA strand breaks and nicotinamide adenine diphosphate depletion [11]. Therefore, the genotoxic effects of arsenic compounds include induction of oxidative stress and inhibition of DNA repair. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids, and carbohydrates, and this leads to a number of physiological disorders [12].

Inorganic arsenic metabolizes to dimethylarsenate which induces DNA damage and DNA single strand breaks as it inhibits repair polymerization [13,14]. The arsenic-mediated DNA protein interaction plays major role in carcinogenesis and induces protein-associated strand breaks which may provide explanation of chromosomal aberrations (CA) [15].

Natural phytochemicals hold a great promise in clinical therapy as they possess no side effects that are usually associated with chemotherapy or radiotherapy [16]. Medicinal plants are used for home remedies and are widely used in developed and developing countries. They are a diverse source of nutrients and bioactive compounds that are valuable for human health [17]. Plants and its products are being used as a medicine since long. Large number of phytochemicals is found in plant food [18]. Having a diet full of vegetables and fruits provide a large number of phytochemicals, antioxidants and nutritive products that boost our health. The natural dietary products such as various fruits, vegetables, and spices, which have drawn a great deal of attention from scientific community and the general public due to their various health-promoting effects including suppression of cancers.

Zingiber officinale (Ginger) is a most widely consumed spice worldwide. It has a long history of use as a herbal medicine for the ailments for vomiting, pain, indigestion, and cold-induced syndromes [19,20]. Ginger is known for its antioxidant, anti-inflammatory, and anticancer activities [21]. The active components of the ginger are thought to be the volatile oils, and various phenolic compounds such as gingerols, shogaols, zingerone, and gingerberols [22,23]. Various authors have demonstrated strong antioxidant properties of ginger. The antigenotoxic action of ginger has been found as one of the possible mechanisms of oxygen free radical scavenging followed by decreased production of ROS [24,25]. Ginger rhizome contains two classes of constituents: (1) The essential oils which give the aroma and (2) the main pungent principles called gingerols [26].

In this study, the aqueous extract of *Z. officinale* (Ginger) was prepared to evaluate its antigenotoxic potential to combat arsenic trioxide-induced genotoxicity in albino mice.

MATERIALS AND METHODS

Animals

Swiss albino mice weighing 20 ± 22 g were procured from Central Research Institute, Kasauli. They were kept and acclimatized to the laboratory conditions for 15 days under optimal conditions of light and temperature. They had *ad libitum* access to tap water. The animals were handled with human care in accordance with the guidelines of the institutional animal ethical committee 107/99/CPCSEA/2013-03.

Chemicals and preparation of extract

Arsenic trioxide was bought from Qualikems Fine Pvt. Ltd., New Delhi. It was dissolved in double distilled water and administered orally to mice. *Z. officinale* (Ginger) was obtained from local market, and aqueous extract was prepared by the method of Ashraf *et al.* [27] and was also administered orally to mice.

Experimental design

The mice were divided into four groups of five mice each. Group I - control animals were given distilled water. Group II - animals were administered an acute dose of 5 mg/kg bw of arsenic orally for 15 days. Group III - animals were given an acute dose of 5 mg/kg bw of arsenic followed by a daily dose of 20 mg/kg bw of ginger for 15 days. Group IV - mice were administered 20 mg/kg bw of ginger orally for 15 days and kept as positive control. Autopsies were done on 15-day post-treatment.

1. The bone marrow cells of albino mice were examined for CA according to the method described by Preston *et al.* [28]. The animals were administered colchicine intraperitoneally for 2 h before dissection. At least 200 metaphases, plates were examined using research microscope with oil immersion lens.
2. Micronuclei (MN): Were evaluated by the procedure given by Elbin *et al.* [29].
3. Statistical analysis: The data were analyzed using Student's t-test using GraphPad by considering $p < 0.001$ as significant and $p > 0.05$ as non-significant.

RESULTS AND DISCUSSION

Arsenic is one of the most important global environmental toxicants. It induces ROS production which is known to affect cell signaling, apoptosis, increase in cytokine production, leading to inflammation and stimulation of more ROS production and mutagenesis, contributing to pathogenesis of arsenic-induced diseases [30].

The major targeting site of free radicals is genetic material carried by the cells. The types of damages include strand breaks (single or double strand breaks), various forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or abasic sites, damage to deoxyribose sugar, as well as DNA protein cross-linkages [31]. These damages can result in inheritable mutations that can yield a cancer in somatic cells or fetal malformations in the germ cells. The involvement of free radicals with tumor suppressor genes and proto-oncogenes recommend their role in the development of different human cancers [32].

Genotoxicity occurs when large number of ROS reacts with both deoxyribose and bases in the DNA causing base lesions and structural breaks. The ROS species also alter the DNA repair mechanism species [33]. Arsenic toxicity causes oxidative

stress. The production of ROS by arsenic is also supported by scientific data, and this mode of action of the metal got acceptance [34,35]. During the present investigation, arsenic trioxide was observed to possess the genotoxic potential as revealed by statistically significant ($p < 0.01$) increase in various types of CA on 15-day post-treatment

in comparison to control group. Who reported that arsenic caused genotoxic and other ill health effects. The chromosomal abnormalities and MN were detected in the interphase cells of human population who were exposed to arsenic.

There are various types of CA which were studied in the four groups at 15-day post-treatment. Data of chromosomal assay were represented in Fig. 1. The frequency of ring chromosome 2 ± 0.32 , loss of definite walls 1.2 ± 0.37 , stickiness 1.2 ± 0.37 , stretching 1.2 ± 0.58 , and fragment 1.6 ± 0.51 in control group and the frequency of CA were lower in comparison to arsenic-treated group. In arsenic-treated group frequency of ring chromosome ring 8.2 ± 0.37 , loss of definite walls 8 ± 0.32 , stickiness 8.2 ± 0.37 , stretching 8.6 ± 0.24 , fragment 8.2 ± 0.37 . Arsenic + *Z. officinalis* frequency of ring chromosome ring 5 ± 0.32 , loss of definite walls 6.6 ± 0.24 , stickiness 6 ± 0.45 , stretching 7.8 ± 0.37 , fragment 5.8 ± 0.58 .

Z. officinalis frequency of ring chromosome ring 3.4 ± 0.4 , loss of definite walls 3.2 ± 0.2 , stickiness 3 ± 0.32 , stretching 3.6 ± 0.51 , fragment 2.6 ± 0.24 . The dose of *Z. officinale* modified the arsenic trioxide-induced changes in MN.

Incidence and CA ring (Graph 1), loss of definite walls (Graph 2), stretching (Graph 3), stickiness (Graph 4) and fragmentation (Graph 5) multiple aberrations to a significant extent as compared to that of arsenic-treated group alone. A similar observation was found in the study of Nirmala *et al.* [36] who have reported the *in vivo* antimutagenic potential of ginger on formation and excretion of urinary mutagens in rats using an *in vitro* assay method.

In the present study, CAs, for example, the chromosomal breaks, ring, and other multiple aberrations such as fragmentation, stretching, stickiness, and loss of definite walls were found to be significantly increased in arsenic-treated group. The studies on human fibroblast cells, leukocytes, lymphocytes, and hamster cells which indicated that the arsenic is highly toxic and caused genotoxic effects on the chromosomes, i.e. CAs and sister chromatid exchange [37]. Various studies done on humans suggest that CAs were maximum in peripheral lymphocytes of humans after both the inhalation and oral exposures. In our study, we observed that CA and the genotoxic effects increase in mice with increase in duration of exposure to arsenic.

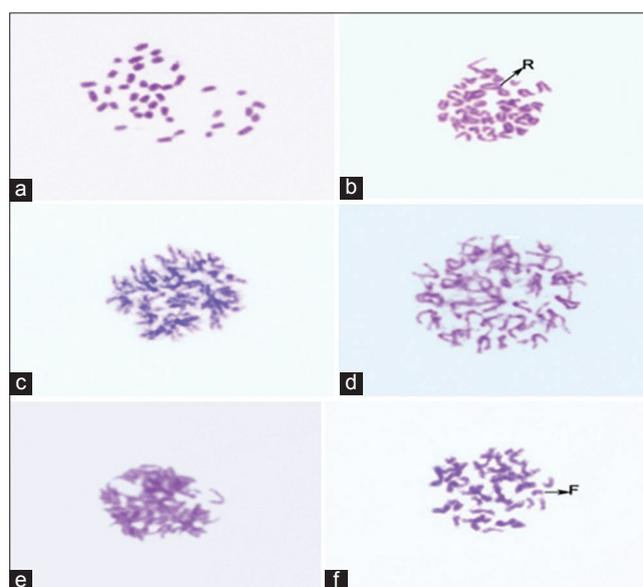
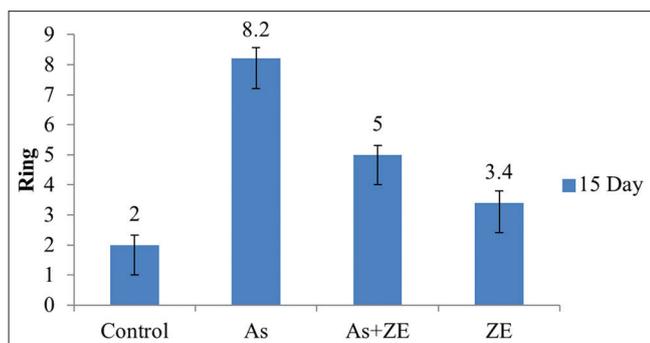
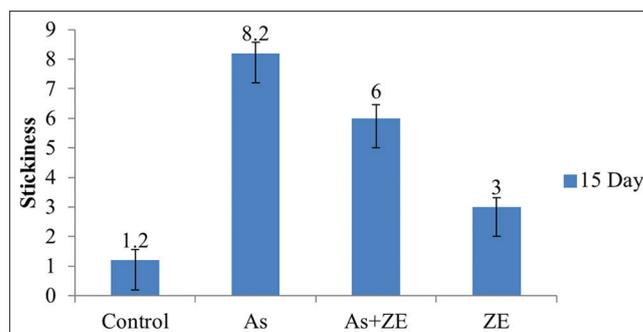


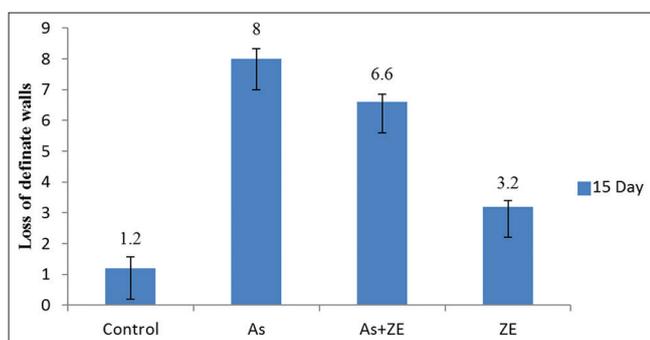
Fig. 1: A photomicrograph of metaphase spread chromosome in bone marrow of albino mice showing the modulatory effect of ginger on arsenic-treated mice for 15-day post-treatment: (a) Control, (b) ring, (c) loss of definite wall, (d) stretching, (e) stickiness, (f) fragment



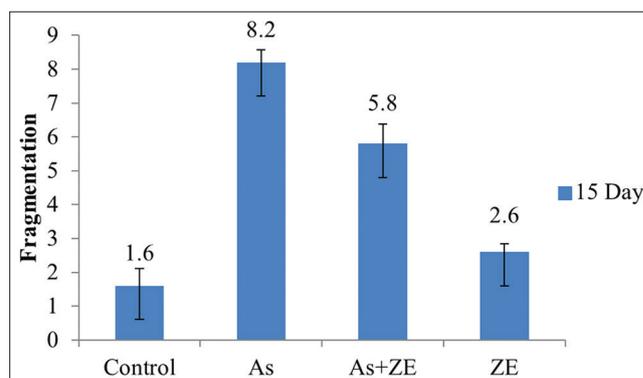
Graph 1: Chromosomal aberrations (ring) in control, arsenic, antioxidant, and positive treated mice at 15-day post-treatment



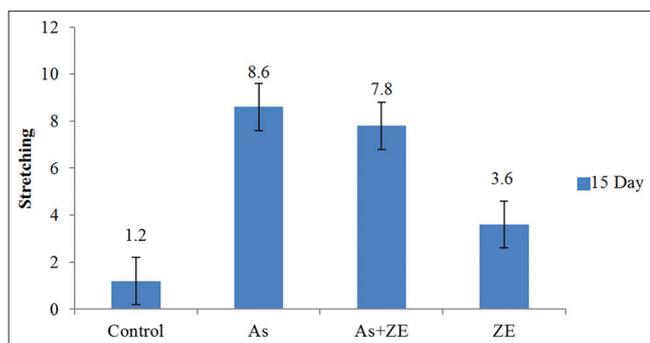
Graph 4: Chromosomal aberrations (stickiness) in control, arsenic, antioxidant, and positive treated mice at 15-day post-treatment



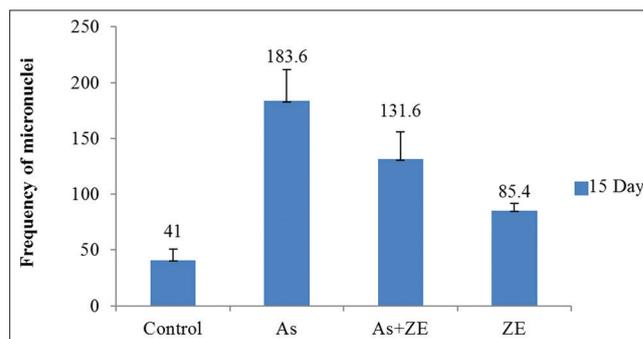
Graph 2: Chromosomal aberrations (loss of definite walls) in control, arsenic, antioxidant, and positive treated mice at 15-day post-treatment



Graph 5: Chromosomal aberrations (fragmentation) in control, arsenic, antioxidant, and positive treated mice at 15-day post-treatment



Graph 3: Chromosomal aberrations (stretching) in control, arsenic, antioxidant, and positive treated mice at 15-day post-treatment



Graph 6: Frequency of micronuclei at 15-day post-treatment

In this study, some of the metaphase plates demonstrated the sticky behavior of chromosomes because they stick to each other at definite places. Some showed the fusion of acrocentric chromosomes that resulted in the formation of metacentric chromosomes and few foci showed telomeric fusion which ultimately formed ring chromosomes. Various chromosomes showed centromeric separation of chromatids as well as chromatid breaks. The fragmented chromosomes were also studied in which arms contained less dense telomeres which confirmed the early loss of genes residing at the telomeric ends and are responsible for the cellular stress including ageing [23].

Z. officinale (Ginger) has been reported as an antioxidant agent [38,39] due to its antioxidant properties and free radical scavenging characteristic and thus it is suited for metal chelation [40,41]. The results of the present study also highlight the beneficial potential of ginger and thus can be used as a nutrient supplement in heavy metal toxicity due to its tremendous antioxidant properties. Gingerol

was very effective in lowering the genotoxic effects [42]. Similarly, Sharif [43] reported genoprotective and hepatoprotective activities of ginger against carbon tetrachloride toxicity. Various studies made with ginger supplementation revealed that ginger acts as antimutagenic agent against carcinogenic activities *in vivo* and *in vitro* studies and is effective in reducing the genotoxic effects and DNA damage [44].

The MN test confirmed chromosomal damage as well as disturbance in mitotic spindle apparatus (clastogenic or aneugenic effect) in polychromatic erythrocytes in bone marrow. MN are cytoplasmic chromatin containing bodies formed in bone marrow erythroblasts. During the maturation process, the erythroblast develops into a polychromatic erythrocyte; the nucleus undergoes extrusion and MN persisting in enucleated cytoplasm can be detected using specific staining processes. The mean value of MN in control group was 41.00 ± 9.74 and in arsenic-treated group was 183.4 ± 27.74 which showed a statistically significant ($p < 0.01$) elevation in comparison to

control group. The frequency of MN in Arsenic+ *Z. officinale* treated group was 131.6±24.06 and in *Z. officinale* was 85.40±6.10 both showed statistically significant elevation in comparison to control group and showed non-significant increase in comparison to arsenic-treated group. The MN frequency in bone marrow of mice coexposed to arsenic and benzopyrene [5].

The rat micronucleus method was used to detect the genetic toxicity *in vivo*. The micronucleus test in rodents (rats and mouse) is available in mammalian test system for detection of CAs [45].

In the present study, the CAs and MN frequency was observed to be significantly increased in arsenic-treated group in comparison to control and ginger-treated group. On the other hand, no significant difference was found in CAs in the group treated with only *Z. officinale* (Ginger) as compared to control group.

The data generated from this present study clearly indicate a significant increase in the MN frequency in arsenic-treated group in comparison to other treated groups; it is confirmed with the work of Dulout *et al.* [46]. Similarly, reported that the elevation in the number of MN frequency in polychromatic and non-chromatic erythrocytes serve as an index of chromosomal damage [47]. According to Patlolla and Tchounwou [48], MN test is more reliable test than CAs or sister chromatid exchange. Previous studies showed that arsenic-induced MN quickly in mice after administration of high doses of arsenic [44,49]. The induction of MN after exposure to As₂O₃ in our study was found to be dose dependent which is in accordance with the earlier published studies [50,51]. Both CAs and MN are genotoxicity markers [52-57]. Although their presence does not lead to adverse effects, their high-level indicates that cell has been exposed to the carcinogens. However, the biochemical alterations modulate the expression of growth control genes which are important in carcinogenesis or apoptosis [58].

CONCLUSION

Thus, in our *in vivo* study, *Z. officinale* (Ginger) possessed antigenotoxic effect along with antioxidant activity in albino mice. The regular consumption could be beneficial to counteract the adverse effects by enhancing antioxidant defense mechanism and neutralizing the toxic effect of ROS generated by the genotoxicants that are present in the environment. More research studies on other genotoxic models are suggested to establish the role of *Z. officinale* (Ginger) against the genotoxicants.

AUTHOR'S CONTRIBUTIONS

Anjali Singh Gill: Interpretation of data, drafting of report, and approval of the version to be published. Dr. Suman Sharma: Interpretation of data, drafting of report, and approval of the version to be published.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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