



# Genotoxicity of sodium arsenite on *Vicia faba* root meristematic cells

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## Abstract

The present study was designed to evaluate the genotoxicity of sodium arsenite by employing the *Vicia faba* root chromosomal aberration assay. The seedlings were treated with different concentrations (0.2, 0.4, 0.6, 0.8, and 1 mg/l) of sodium arsenite. In addition to the cytogenetic assay, effect of arsenic on biochemical parameters such as total protein content and antioxidative enzymes [Sodium dismutase (SOD) and Guaiacol peroxidase (POD)] were also studied in 7-day old *Vicia faba* seedlings. Results demonstrated that metal stress significantly induced chromosomal aberrations and exhibited cytotoxicity by lowering the mitotic index from  $9.37 \pm 0.05$  to  $3.73 \pm 0.01$ . Biochemical analysis showed a significant decrease in total protein content and increased activity of antioxidative enzymes under heavy metal stress as compared to control. The present investigations not only provide insights into arsenic genotoxicity but also support the use of various arsenic induced genotoxic characteristics as toxicity indicators in plants.

**Keywords** Arsenite · *Vicia faba* · Mitotic index · Chromosome aberrations · Antioxidant enzymes

## Introduction

Arsenic (As) is a harmful metalloid which can exist in soil and water in different organic and inorganic forms is one of the major environmental pollutants contaminating water and soil through industrial applications such as mining, coal combustion, agricultural practices, semiconductors, etc. and natural processes [2, 35]. An inorganic form of arsenic consists of arsenite and arsenate. The arsenite form is considered to be the most toxic form [43]. Arsenic is an element which is not needed for plant growth. As enters plants and interferes with their metabolic activities, causing physiological and morphological defects as well as plant growth suppression [24–26, 30]. The augmented production of reactive oxygen species (ROS) is considered to be the main metabolic effect of As toxicity [27, 40]. ROS damage essential

biomolecules such as lipids, proteins, carbohydrates, and DNA [38, 39]. DNA damage from genotoxic substances can cause chromosomal aberrations (CA), micronuclei, and DNA breaks, all of which can be used as genotoxic markers [31]. Arsenic has been demonstrated to be genotoxic in a wide variety of experiments and induces genetic abnormalities both in vitro and in vivo systems [3, 17, 19, 34].

Higher plants provide an effective genetic system for evaluating the damaging effects of chemicals. A variety of plant systems, including *Allium cepa*, *Vicia faba*, *Arabidopsis thaliana*, and *Hordeum vulgare* have been investigated for analyzing the mutagenic activity of chemicals [37]. The plant bioassays are easy and economical and can be used in clastogenic screening as the plants can sense genotoxicity more efficiently and promptly than animal assays [44]. Root tip chromosomal aberration assay has been extensively used for studying chemical induced chromosomal aberrations as contains a high percentage of actively dividing cells. *Vicia faba* has the benefit of having six pairs of relatively large chromosomes with stable karyotype. These chromosomal features of *Vicia faba* helps in easy identification of mitotic stages and chromosomal alterations. Genotoxic end points consist of upsurge and decline in mitotic index as compared to control [4]. The material is readily available throughout the year; it is low-cost and simple to cultivate and handle. It also does not require any sterile conditions to grow [21]. The

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roots may be prime subjects and first defense organs against heavy metal stress [42]. In this study, *Vicia faba* seedlings were used as test material. The objective of the current study was to investigate the genotoxic effects in roots of *Vicia faba* seedlings grown in different concentrations of sodium arsenite and to further screen the defense responses in the roots against arsenic toxicity.

## Materials and methods

### Experimental material

In the present study, *Vicia faba* was used as a plant system to check the genotoxicity of sodium arsenite (CAS no. 7784-46-5) using *Vicia faba* root chromosomal aberration assay. The certified and disease free seeds of *Vicia faba* L. (Vikrant - VH-82-1) were procured from Department of Plant Breeding, Hisar, Haryana.

### Morphological parameters

#### Root growth inhibition test

Root growth inhibition test is simple, reliable, and easy to perform and provides a better idea about the toxicity of the experimental samples. This test is generally used to calculate the  $EC_{50}$  on the basis of root length of the plants treated with different concentrations of the test samples. *Vicia faba* seedlings were soaked in tap water for 24 h and were then allowed to germinate in different concentrations of sodium arsenite (0, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 mg/l) for 96 h. Tap water used as a negative control. The test was performed in 3 replicates. The test solutions were replaced with fresh solutions after every 24 h. The three seedlings with longest roots were selected from each replicate and the root length was measured.  $EC_{50}$  (the effective concentration of a chemical producing 50% of the total effect in comparison to control) was calculated for the test samples.

### Genotoxicity investigations

#### *Vicia faba* seed germination

The seeds of *V. Faba* were surface sterilized and washed thoroughly in distilled water for three times. About 25–30 seeds were soaked in distilled water for 24 h. Seed coat was removed carefully and seeds were allowed to germinate in autoclaved petri-plates containing moist cotton for 4 days at  $23 \pm 1$ . When newly emerged primary roots reached 3.0–5.0 cm in length, the root tip (5 mm) was cut off. The seedlings were then incubated in aerated tap water at  $22 \pm 2$  °C to allow the development of lateral roots. After

4 days, the lateral roots (1–2 cm) were used for treatment with sodium arsenite.

### Treatment with sodium arsenite

A stock solution of sodium arsenite (2 mg/l) was prepared just before use. The germinated seedlings of *V. faba* were treated with various concentrations of sodium arsenite viz. 0.2, 0.4, 0.6, 0.8 and 1 mg/l for 3 h at  $22 \pm 2$  °C in the dark.

### Fixation of root tips and staining

After the treatment, the roots were cleaned thoroughly with tap water, harvested and fixed in Farmer's fluid (glacial acetic acid: ethyl alcohol in the ratio of 1:3) for 24 h. These were then shifted to 70% ethanol and stored at 4 °C. The slides were prepared by hydrolyzing the root tips in 1N HCl with intermittent heating for 1 min and then treated with 1N HCl and aceto-orcein stain in ratio of 1:9. The root tips were then warmed for 2–3 min with intermittent heating, covered and then kept for 30 min. The root tips were placed on slide and squashed in 45% (v/v) glacial acetic acid and tapping the coverslip with a matchstick and mounted with DPX.

### Scoring of slides

The slides were observed for various types of chromosomal aberrations. About 750 dividing cells from 7–8 root tips (~ 100 cells/root tip) were scored. Photomicrographs were taken with the help of a digital camera fixed on microscope (Olympus) connected to a computer to transfer images. The physiological type of chromosomal aberrations scored were C-mitosis, delayed anaphase/s, stickiness and vagrant/s. The clastogenic chromosomal aberrations scored were chromatin bridge/s, chromosomal break/s and ring chromosome.

### Calculations

The mitotic index (MI) was calculated as the number of dividing cells per 100 cells for each treatment. Chromosomal aberrations were scored and the results were tabulated.

$$MI = \frac{\text{Total no. of dividing cells}}{\text{Total no. of cells}} \times 100$$

Percent aberrant cells were calculated by:

$$\text{Percent aberrant cells} = \frac{\text{Number of aberrant cells}}{\text{Number of dividing cells}} \times 100$$

## Biochemical investigations

### Preparation of *Vicia faba* extracts

1 g of fresh roots of germinated seedlings of *Vicia faba* treated with various concentrations of sodium arsenite (0.2, 0.4, 0.6, and 0.8 mg/l) were crushed and homogenized using pestle and mortar in 3 ml of pre-chilled potassium phosphate buffer (100 mM, pH 7.0) under ice-cold conditions. The homogenate was centrifuged at 10,000g at 4 °C for 20 min. The supernatant was collected for total protein and antioxidant enzyme analyses.

### Estimation of protein content

Total protein content of the seedlings was quantified by the method of Bradford [5] using bovine serum albumin as standard. 2 ml of Bradford reagent was added in 150 µl distilled water and 50 µl sample extract in each test tube and the absorbance was taken at 595 nm using a spectrophotometer (Systronics 2202 UV–Vis spectrophotometer, Gujarat, India). A graph of absorbance versus concentration for standard solution of protein was plotted and the amount of protein was calculated from the graph. The amount of protein was expressed as mg/g f.w.

### Estimation of antioxidative enzymes

#### Superoxide dismutase (SOD, EC 1.15.1.1)

Superoxide Dismutase activity was assayed according to the methodology of Kono [23] with slight modifications. For total SOD assay, 3.0 ml reaction mixture contained 50 mM sodium carbonate buffer (pH 10.2), 96 µM NBT, 20 mM hydroxylamine hydrochloride, 0.6% Triton X-100 and 70 µl of the enzyme extract. The absorbance was recorded spectrophotometrically (Systronics 2202 UV–Vis spectrophotometer, Gujarat, India) at 540 nm for 2 min.

#### Guaiacol peroxidase (POD, EC 1.11.1.7)

Guaiacol Peroxidase was assayed according to the method given by Putter [36]. The activity of the peroxidase can be determined by the decrease of H<sub>2</sub>O<sub>2</sub>, or the hydrogen donor or the formation of oxidized compound. The reaction mixture (3.0 ml) contained 50 mM phosphate buffer (pH 7.0), 20 mM guaiacol solution, 12.3 mM H<sub>2</sub>O<sub>2</sub> and 100 µl enzyme extract. The POD activity was assayed by measuring the absorbance at 436 nm and using an extinction coefficient of 26.6 mM<sup>-1</sup> cm<sup>-1</sup>.

## Statistical analysis

Each experiment was carried out in triplicates. The data was subjected to one way ANOVA and expressed as the mean ± standard error. The significance level was checked  $p \leq 0.05$ .

## Results

### Morphological parameters

#### Root growth inhibition test

The estimated EC<sub>50</sub> (the effective concentration of a chemical that produces 50% of the total effect when compared to the control) of *Vicia faba* seedlings exposed to sodium arsenite was 0.8 mg/l. The highest concentration of sodium arsenite (2 mg/l) showed 21.70% root growth as compared to control (tap water) whereas minimum tested concentration (0.2 mg/l) showed 95.73% root length as compared to control. The root length decreased as the concentration of sodium arsenite increased. One way Anova analysis showed F-ratio = 396.72 and HSD = 0.629 (Fig. 1).

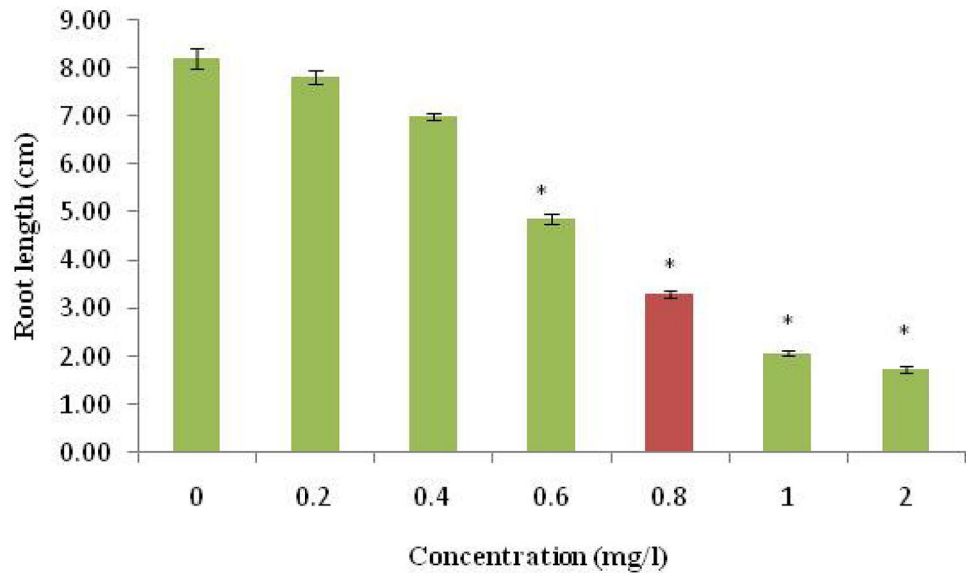
### Genotoxicity

The mitotic index (MI) was found to decrease significantly as compared to control. NaAsO<sub>2</sub> significantly induced chromosomal aberrations and exhibited cytotoxicity by lowering the mitotic index from  $9.37 \pm 0.05$  to  $3.73 \pm 0.01$ . NaAsO<sub>2</sub> induced more physiological aberrations (20.93%) at 0.8 mg/l as compared to clastogenic aberrations (9.87%). Physiological aberrations included c-mitosis, delayed anaphase/s, vagrant/s and stickiness while clastogenic aberrations such as chromosomal break/s, chromatin bridge/s and ring chromosome were observed (Table 1, Fig. 2). One way Anova analysis showed F-ratio = 33.683 and HSD = 6.712.

### Total protein content

The total protein content decreased in the seedlings treated with arsenic metal solution as compared to untreated seedlings (Fig. 3). Protein content was decreased from 107.2 (mg/g f.w.) in control seedlings to 38.83 (mg/g f.w.) in seedlings treated with 0.8 mg/l arsenic. The protein content decreased with increasing concentration of sodium arsenite. One way Anova analysis showed F-ratio = 10.352 and HSD = 40.134.

**Fig. 1** Root length of *Vicia faba* exposed to different concentration of sodium arsenite for 96 h. CN=Control (tap water). \* Statistically significant at  $p < 0.05\%$  level (compared to control)



**Table 1** Chromosome aberrations in *Vicia faba* root cells exposed to sodium arsenite

Concentration (mg/ml)	MI ± SE	Physiological aberrations (PA)					Clastogenic aberrations (CA)				PA + CA (%)
		Cm	Da	St	Vg	Total PA (%)	Bg	Bk	Rc	Total CA (%)	
0	9.37 ± 0.05	12	6	8	–	3.47	3	13	–	2.13	5.60
0.2	4.23 ± 0.02*	31	22	25	–	10.40	12	33	–	6.00	16.40*
0.4	4.11 ± 0.03*	30	27	34	3	12.53	11	41	–	6.93	19.47*
0.6	3.98 ± 0.05*	41	25	47	6	15.87	8	45	–	7.06	22.93*
0.8	3.78 ± 0.01*	52	44	52	9	20.93	15	58	1	9.87	30.80*
1.0	3.73 ± 0.01*	38	5	56	–	13.20	4	63	–	8.93	28.93*

PA-Physiological aberrations; Cm-C-mitosis; Da-Delayed anaphase; Vg- Vagrant/s; St-Stickiness; CA- Clastogenic aberrations; Bg-Bridge/s; Bk-chromosomal break/s, Rc-Ring chromosome/s

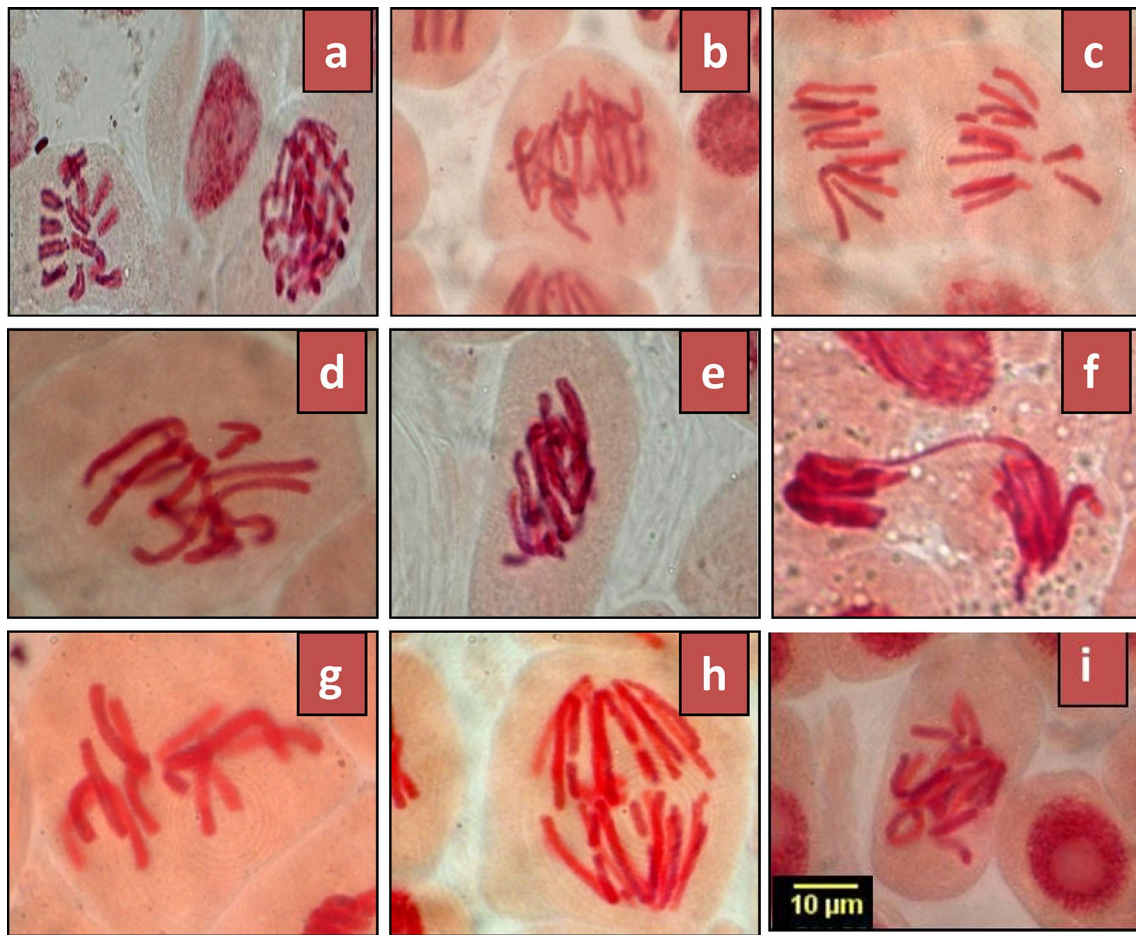
Data shown are mean ± SE. Significant differences from control are indicated as \* at  $p < 0.05\%$  level

## Antioxidant enzyme activities

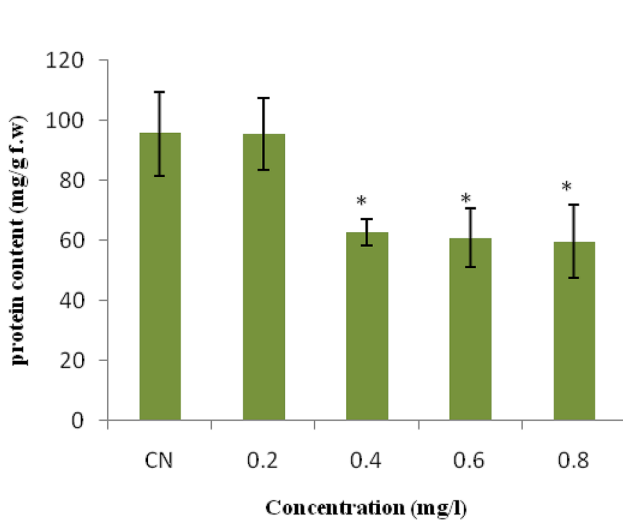
Specific activity of superoxide dismutase was observed to increase considerably under metal stress with increasing concentrations of sodium arsenite. Maximum value of SOD (0.306 unit activity/mg protein) was observed at 0.8 mg/l arsenic as compared to untreated control seedlings ( $0.069 \pm 0.007$  unit activity/mg protein) (Fig. 4). One way Anova analysis showed F-ratio=6.213 and HSD=0.177 for SOD activity. The activity of POD increased with increasing concentration of sodium arsenite. The activity of POD was more at 0.8 mg/l arsenic (0.014 unit activity/mg protein) as compared to control (0.002 unit activity/mg protein) (Fig. 5). For POD, One way Anova analysis showed F-ratio=6.286; HSD=0.0085. Results were found to be statistically significant at the 0.8 mg/l treatment for both enzyme activities.

## Discussion

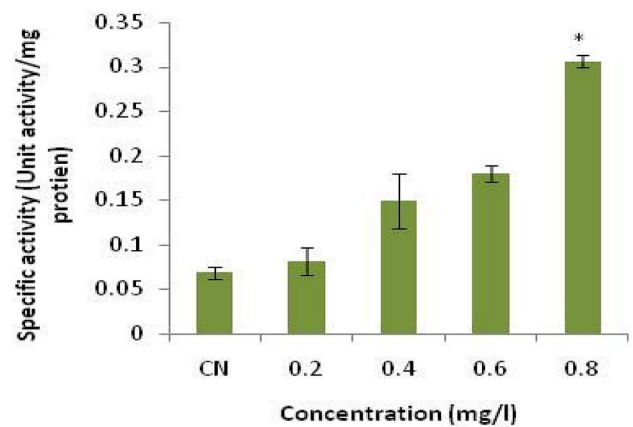
Large regions are exposed to heavy metal pollution due urbanization and industrialization [33]. Although many heavy metals when present in trace amounts are essential for various metabolic processes in organisms but at high concentration, they tend to create physiological stress due to generation of free radicals [18]. Oxidative damage is caused by an imbalance between free radical generation and the antioxidant defence system [9]. Among different heavy metals, arsenic is possibly the most abundant pollutant having complex metabolism and has been classified as a potent human carcinogen. Exposure to arsenic has been linked with augmented risk of various types of cancers, skin diseases, ischemic heart diseases, neurological effects and teratogenicity [12]. Vogt and Rossman [41] reported that As (III) can induce genetic effects such as chromosomal aberrations,



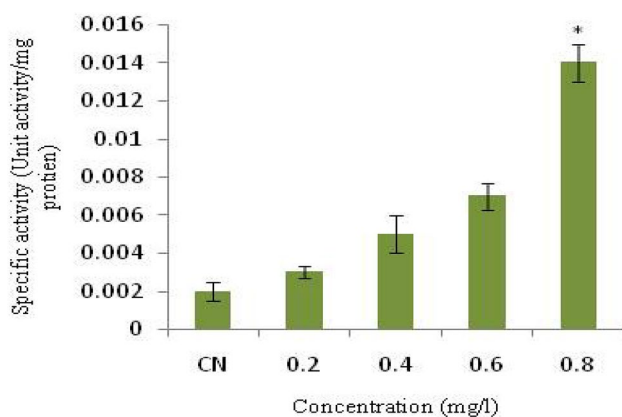
**Fig. 2** Effect of sodium arsenite on mitosis of *Vicia faba* root cells. **a** C-mitosis, **b** delayed anaphase, **c** vagrant at anaphase, **d** vagrant at metaphase, **e** stickiness, **f** chromatin bridge, **g** break at metaphase, **h** break at anaphase, **i** ring chromosome



**Fig. 3** Effect of sodium arsenite on the protein content of *Vicia faba* seedlings. CN=Control (tap water). \* Statistically significant at  $p < 0.05\%$  level (compared to control)



**Fig. 4** Effect of sodium arsenite on the SOD activity of *Vicia faba* seedlings. CN=Control (tap water). \* Statistically significant at  $p < 0.05\%$  level (compared to control)



**Fig. 5** Effect of sodium arsenite on the POD activity of *Vicia faba* seedlings. CN=Control (tap water). \* Statistically significant at  $p < 0.05\%$  level (compared to control)

micronuclei formation etc. and also recognized to inhibit DNA repair. Arsenicals can cause damage to DNA by inhibiting the enzyme activity involved in DNA repair. Genetic abnormality without effective DNA repair causes certain types of mutations and eventually initiates carcinogenesis.

As shown in Table 1, sodium arsenite significantly induced chromosomal aberrations and exhibited cytotoxicity by lowering the mitotic index from  $9.37 \pm 0.05$  to  $3.73 \pm 0.01$ . Sodium arsenite induced physiological aberrations *viz.* c-mitosis, delayed anaphase/s, vagrant/s and stickiness and clastogenic aberrations *viz.* chromosomal break/s, chromatin bridge/s and ring chromosome. Sodium arsenite induced more physiological aberrations (20.93%) at 0.8 mg/l as compared to clastogenic aberrations (9.87%). The different types of chromosomal alterations could be induced either by disruption of spindle, C-mitosis, stickiness, by disorientation and multipolarity at anaphase [15]. Similar results were obtained in a study conducted by Bandyopadhyay [1] in *Allium cepa* treated with sodium arsenate and sodium arsenite. It was found that the upsurge in chromosomal aberrations and reduction in the mitotic index was directly related to the concentration of the mutagen and duration of exposure. Pandey and Upadhyay [33] also reported the clastogenic effect of arsenic on root meristem cells of *Vicia faba* L. The mitotic index declined gradually as the concentration of arsenic metal increased.

Heavy metals have an aneugenic impact on C-mitosis, inhibiting spindle formation and increasing chromosome adhesion owing to aberrant chromosomal proteins [33]. Sticky nature of chromosomes might be due to the disturbance in nucleic acid metabolism of the cell [6]. Stickiness might also be caused by improper folding of chromosomal fibres into a single chromatid, resulting in fibre intermingling and chromosomes becoming linked to one other via sub-chromatid bridges [22]. Delayed anaphase occurs

when two anaphasic groups of chromosomes lie close to each other near equatorial plate. Fiskesjo [11] also reported the occurrence of delayed anaphase in *Allium cepa* after treatment with mercury and selenium. The start of vagrant chromosomes leads in the division of an uneven number of chromosomes in the daughter cells, resulting in the creation of unequally sized or irregularly shaped nuclei during interphase [8]. Chromosomal breakage was most prominent clastogenic aberration observed in the present study. The chromosomal breakage is thought to involve the DNA responsible for the chromosomes' linear continuity and may be caused by incomplete or incorrect DNA repair [10]. Gormurgen [14] attributed the formation of chromosomal bridges to the failure of free anaphase separation, uneven translocation or inversion of chromosome fragments and concluded that bridge formation causes structural chromosome mutations. Yildiz et al. [45] accredited the induction of bridges to chromosome break, stickiness, breakage and reunification of the broken ends. Distal breaking of the short and long arm of a chromosome causes rejoining at the ends leading to the formation of ring chromosomes [13].

Proteins are a critical component of the cell that is readily affected under environmental stress situations. As a result, any changes in these might be regarded as a key sign of oxidative stress in plants. The total protein concentration was found to decrease with increasing metal treatment. The decrease in total protein content caused by heavy metal stress might be ascribed to increased protease activity, which speeds up the protein breakdown process [32]. Our results are in concordance with Latif [1] who also reported a significant decrease in total protein content with increasing concentration of nickel sulphate in *Raphanus sativus* L. Hamid et al. [16] also reported decline in total protein content in *Phaseolus vulgaris* with different treatments of lead. Arsenic is believed to have caused lipid peroxidation and protein fragmentation as a result of the harmful effects of reactive oxygen species, resulting in lower protein content [20].

Arsenic toxicity can result in an increase in the generation of ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. Cells can defend themselves from oxidative damage by using enzymatic and non-enzymatic systems such as SOD, CAT, POD, or glutathione [7]. SOD and POD are significant antioxidant enzymes that check the buildup of various ROS in the aerobic cells [21]. The observations in the present study revealed that application of arsenic metal solution to *Vicia faba* resulted in increased activity of anti-oxidative enzymes *viz.* Superoxide dismutase (SOD) and Guaiacol peroxidase (POD), which is consistent with Lin et al. [29] who reported the enhanced SOD and POD activity with increasing arsenate concentrations in leaves of *Vicia faba*. The increase in SOD activity suggested that arsenate exposure caused the production of  $O_2^{\cdot-}$ , which increased the biosynthesis of SOD while increase in the activity of

POD specifies the buildup of H<sub>2</sub>O<sub>2</sub> in plant tissues which may have stirred the biosynthesis of POD. The increased in activity of SOD and POD was a part of a damage response to stress induced by sodium arsenite.

## Conclusion

Arsenic compounds can cause damage to cells through generation of free radicals and ROS such as superoxide, hydroxyl radicals, hydrogen peroxide during their metabolism in the cells and also induces chromosome aberrations. The present results showed that sodium arsenite is a clastogenic and genotoxic agent in *V. faba* root tip chromosomal aberration assay. These findings clearly reveal that application of sodium arsenite reduced total protein concentration while increasing the activity of antioxidative enzymes (SOD and POD).

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**Availability of data and material** Not applicable.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Consent to participate** Not applicable.

**Consent for publication** All the Authors provide their consent for publication.

**Ethics approval** Not applicable.

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