



Antimutagenic Effects of Garlic and Turmeric Extracts on Chromosomal Aberrations in *Allium Cepa* Root Tip

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Garlic and Turmeric have been used in the preparation of food in India since the Vedic period. Apart from their known uses in food preparation, they are also used in Ayurvedic medicine and they show antimutagenic and antigenotoxic properties. In the present study, antimutagenic effects of garlic and turmeric extracts have been evaluated using chromosomal aberrations in *Allium cepa* root meristem cells. The antimutagenic extracts of garlic and turmeric were used against mutagenic chemicals such as sodium azide, sodium benzoate, sodium citrate, citric acid, potassium metabisulphite and urea. The root tip cells were treated with the above-mentioned chemicals for 24 hours and prior to this procedure, the root tips were pre-treated by garlic and turmeric extracts for 14 hours. These mutagens were found to induce chromosomal aberrations like chromosomal

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bridges, laggards, nuclear lesions, stickiness, and chromosomal fragments, also they were found to affect the mitotic index of the onion root tips. The total number of aberrations was significantly decreased in onion root tip cells when pre-treated with garlic and turmeric extract.

Keywords: *Allium cepa*; chromosomal aberrations; mutagens; antimutagens; genotoxicity.

1. INTRODUCTION

A mutagen is any substance causing permanent changes in the genetic material. Chromosomal aberration is an abnormality where there are changes in the number or the structure of chromosomes often caused by a mutagen. There are distinct ways in which the damage caused by such mutagens can be prevented or reduced up to a certain extent. Any chemical substance that can intrude into DNA repair or inhibit the metabolism of mutagens can be called an effective antimutagen.

In this study, six chemical mutagens were used. Sodium azide (NaN_3) is a major mutagen as it is known to have cytotoxic potential and is used in medicine, agriculture, as a preservative etc. It works by inhibiting protein synthesis and DNA synthesis at concentrations around 200-300 $\mu\text{g/ml}$ [1]. Citric Acid ($\text{C}_6\text{H}_8\text{O}_7$) is a naturally occurring organic compound found in many plants. It is used in insecticides, and disinfectants, to preserve and flavour foods, beverages etc. It is known to cause DNA damage and chromosomal aberrations like anaphase bridge, and c-mitosis [2,3]. Sodium Citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$) is the sodium salt of citric acid. It is used as a food additive, preservative, flavouring agent and as an acidity regulator. Till now, no study has been carried out on the cytological effects of citric acid in plant systems, despite it being commonly used [3]. Potassium metabisulphite ($\text{K}_2\text{S}_2\text{O}_5$) is a white and crystalline powder having a pungent type of odour. It is mainly used as a preservative in food like pickles, juice and in the brewing industry. It has been found to be mitotoxic as the mitotic index decreases with the increase in the concentration of the food preservative [4,5]. Urea $\text{CO}(\text{NH}_2)_2$ is an organic compound also called carbamide. It is used as a fertilizer, and animal feed additive, in the manufacturing of plastics and drugs. It is known to have cytotoxic and genotoxic potential and causes chromosomal aberrations like laggard, sticky chromosomes etc. [6]. Sodium benzoate ($\text{C}_7\text{H}_5\text{NaO}_2$) is the sodium salt of benzoic acid. It is mainly used as a food additive and in preserving processed food and beverages like jams, juice, soda, pickles etc. to extend the

shelf life. It inhibits DNA synthesis and causes chromosomal aberrations like anaphase bridge, laggards, reduction in mitotic division, and c-mitosis [3,7].

Allium cepa is the plant model that was used because its root tip contains meristematic cells responsible for the rapid growth of the onion. It is known to be a popular medium to study mitosis.

The antimutagens used in this study are the extracts of garlic and turmeric. Because of the biologically active component, allicin and its derivatives, garlic has been used in medicine to treat a variety of diseases (Tesfaye, A. 2021). Garlic has antioxidant properties due to which it is also antigenotoxic in nature [8,9]. Turmeric has an active component curcumin due to which it is used in medicine. Many studies show that turmeric has antigenotoxic and antimutagenic properties [1]. Although the antimutagenic potential of garlic and turmeric has been extensively studied and well documented, there is no report on the biological effects of garlic and turmeric in plant test systems (Ragunathan, 2007). The present study was designed to investigate the action of garlic and turmeric against chemical mutagens inducing chromosomal aberrations like laggards, sticky chromosomes, and nuclear lesions in *Allium cepa* root meristem cells [1].

2. MATERIALS AND METHODS

Allium cepa is the plant model used for the following experiment. Chemical mutagens that were used are sodium azide (200 $\mu\text{g/ml}$), citric acid (100 $\mu\text{g/ml}$), sodium citrate (100 $\mu\text{g/ml}$), potassium metabisulphite (10 $\mu\text{g/ml}$), urea (100 $\mu\text{g/ml}$) and sodium benzoate (100 $\mu\text{g/ml}$). Antimutagenic extracts of garlic and turmeric were prepared with the concentration of 0.1g/mL and 0.092g/mL dissolved in methanol respectively.

Healthy onion bulbs were grown in water for 48 hours in three sets at room temperature. From which one of the sets of the onion bulbs was transferred into the mutagenic solutions for 24

hours. The mutagenic solution's root tips were cut, fixed, stained and observed under the light microscope. The second set was transferred into antimutagenic extracts for 14 hours and after that, it was transferred into the mutagenic solutions for 24 hours. After this treatment, the onion root tips were cut, fixed, stained and observed under the light microscope. The third set was kept as a controlled group grown in water and also observed under a light microscope [1].

Slide Preparation: The apex of the onion root was trimmed and placed inside a watch glass containing 1N HCl. With the help of forceps, the root tip in the watch glass was heated over a spirit lamp and kept aside for 10 minutes. After this, the root tip was washed and transferred to another watch glass having acetocarmine stain. After letting it stain for about 20 minutes, the root

tip was washed and placed on a microscopic slide. It was mounted with glycerine and covered with a cover slip. Gentle force was applied on the coverslip to prepare a squash of the root tip. The slide was observed under the light microscope at a magnification of 10x then shifted to 45x with the help of an objective lens. This procedure was common for all the onion root tips of the test and controlled sets for the observation of mitotic stages of cells.

The mitotic index (MI) for genotoxicity evaluation was calculated by dividing the number of cells undergoing mitosis by the total number of cells (Auti, 2010).

$$MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells}} \times 100$$
$$= \frac{\text{Number of cells in prophase+metaphase+anaphase+telophase}}{\text{total number of cells}} \times 100$$



Fig. 1. Controlled set



Fig. 2. Test set - chemical mutagenic treatment



Fig. 3. Test Set – Garlic extract treatment



Fig. 4. Test set – turmeric extract treatment

3. RESULTS

The mitotic index (MI) was calculated, where the mitotic index of the controlled onion root tips was found to be 7.5% indicating no abnormalities. The mutagenic onion root tips had the following mitotic index: sodium azide-2%, citric acid-2.1%, sodium citrate-2.2%, urea-2.4%, sodium benzoate-2.6% and potassium metabisulphite-2.7% thus indicating disturbances in cell division causing chromosomal aberrations. Further, the mitotic index of the onion root tips cells pre-treated with garlic and turmeric extract was 4.5% and 4.7% respectively. The highest mitotic index was observed in the controlled set.

All the chemical mutagens reduced the mitotic index of the onion root tip cells. The pre-treatment of the onions with garlic and turmeric extracts was found to be effective as the mitotic index was high when compared to the MI of the mutagens.

4. DISCUSSION AND ANALYSIS

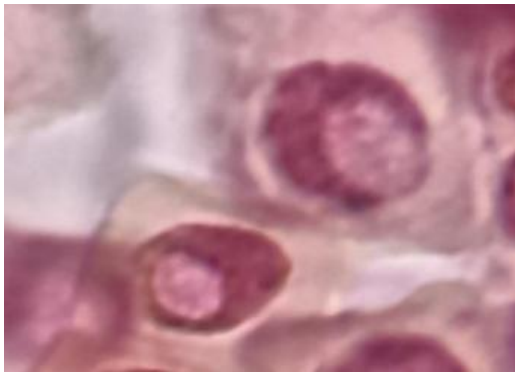
The *Allium* test is generally used to determine the genotoxic or cytotoxic effect of different chemicals. The results of this test permit an estimation of the genotoxicity and mutagenicity of various chemicals that have a direct or indirect influence on living organisms and an estimation of the effects of antimutagens (Garlic extract and Turmeric extract) on the chromosomal aberrations caused by the mutagenic chemicals [2].

The mutagenic chemicals used in this experiment caused different chromosomal aberrations in the onion root tip meristem cells. Sodium citrate induced chromosomal aberrations like c- mitosis (Fig. 11), micronucleus (Fig. 7), laggard (Fig. 12), anaphase bridge (Figs. 8, 9) and chromosomal breaks (Fig. 10). Similar results were observed in other experiments where sodium citrate was used as a mutagen

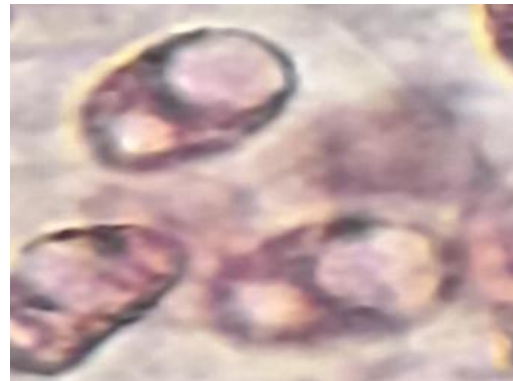
[3,4]. Sodium benzoate caused aberrations like c-mitosis (Fig. 11), stickiness (Fig. 13), clumping of chromosomes and disturbance in the spindle formation. Such types of aberrations were also detected by Khanna, [4,7]. Citric acid was responsible for laggard formation (Fig. 12), chromosomal bridge (Fig. 9), stickiness (Fig. 13) and c-mitosis (Fig. 11). Similar abnormalities were also found by Yılmaz, [2]. Just like sodium citrate, sodium benzoate and citric acid, many other chemicals and food preservatives have been recorded to inhibit mitosis [10]. Reduction in the mitotic index could be due to a hindrance in DNA synthesis or a blocking in the G1, S and G2-phase of the cell cycle, restricting the cell from entering mitosis [11]. The occurrence of C-mitosis in the onion root tip cells indicates inhibition of spindle formation similar to the observation recorded by Kostadinova, [7]. Such c-mitotic cells were also observed to be induced by the treatments with different other preservatives [12]. The chromosomal bridge in the cells treated with sodium citrate and citric acid could have been formed by the breakage and fusion of chromosomes [2]. The appearance of micronuclei could be the outcome of acentric

fragments or laggards that fail to assimilate into either of the daughter nuclei during the telophase stage of the mitotic cells [13]. Similarly, micronuclei were recorded in several experiments following the treatment with different food additives [3,5]. Chromosome stickiness is regarded as a physiological effect which has been recognized to affect the proteins of the chromosomes. Stickiness of chromosomes in the different stages of mitosis reflects highly toxic effects, mostly of an irreversible type which apparently leads to cell death. Türkoğlu, Ş. [3] These results were in accordance with the results of many other research experiments [12]. Laggards must have been formed because of the failure of the chromosomes to get attached to the spindle fibre and to move to either side of the poles [4]. Such lost fragments were unable to participate in the further process of mitosis [3]. Sodium citrate and citric acid can be called clastogens since they induced chromosomal breakage and formation of laggards. The action of such chemicals or preservatives on chromosomes is widely considered to involve an action on the DNA molecule of the plant system.

Chromosomal aberrations:



(Fig. 5)



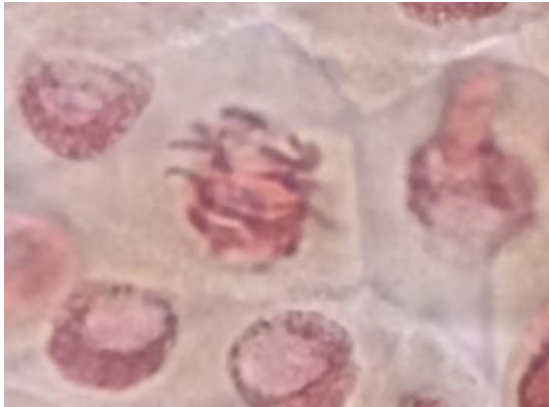
(Fig. 6)



(Fig. 7)



(Fig. 8)



(Fig. 9)



(Fig. 10)



(Fig. 11)



(Fig. 12)



(Fig. 13)

(Fig. 5) & (Fig. 6) – nuclear lesion, (Fig. 7) – micronucleus, (Fig. 8) & (Fig. 9) – anaphase bridge with laggard, (Fig. 10) – chromosomal break, (Fig. 11) – c-mitosis in metaphase, (Fig. 12) – laggard, (Fig. 13) – sticky chromosome

Potassium metabisulphite induced chromosomal aberrations like anaphase bridge (Figs. 8, 9), laggards (Fig. 12) and chromosomal break (Fig. 10). Similar results were observed in the other experiment. Haq et al (2016) suggested that chromosome bridges in the onion root cells might have been caused due to agglutination of

chromosomes [14]. Chromosome agglutination is a predictor of cell deaths, because it has an irreversible effect, inhibiting the formation of the division spindle, and leads chromosomes to stick together, unequal distribution and the creation of anaphase bridges by the fusion of chromosomes or chromatids [15].

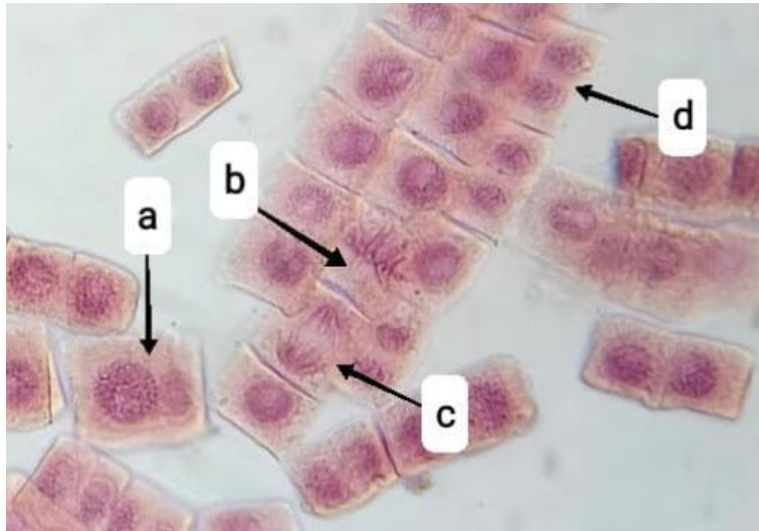


Fig. 14. Controlled Set: [a] normal prophase, [b] normal metaphase, [c] normal anaphase, [d] normal telophase

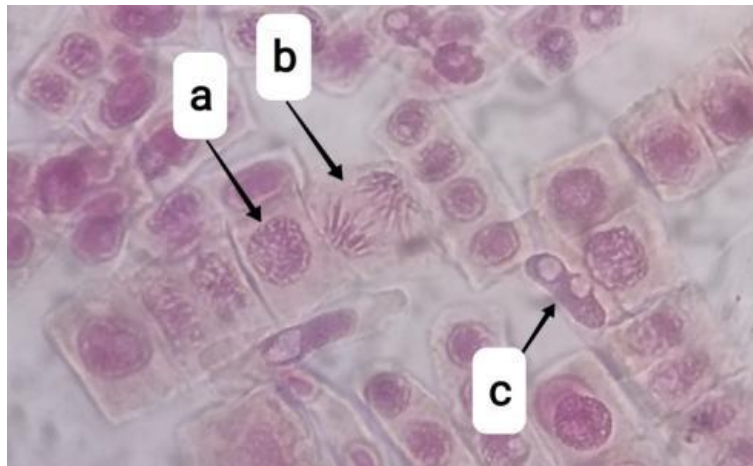


Fig. 15. Test Set: Garlic extract – [a] normal prophase, [b] normal anaphase, [c] nuclear lesion

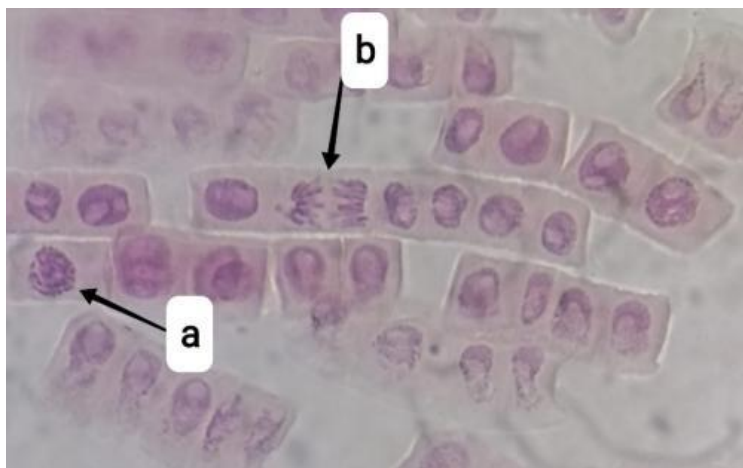
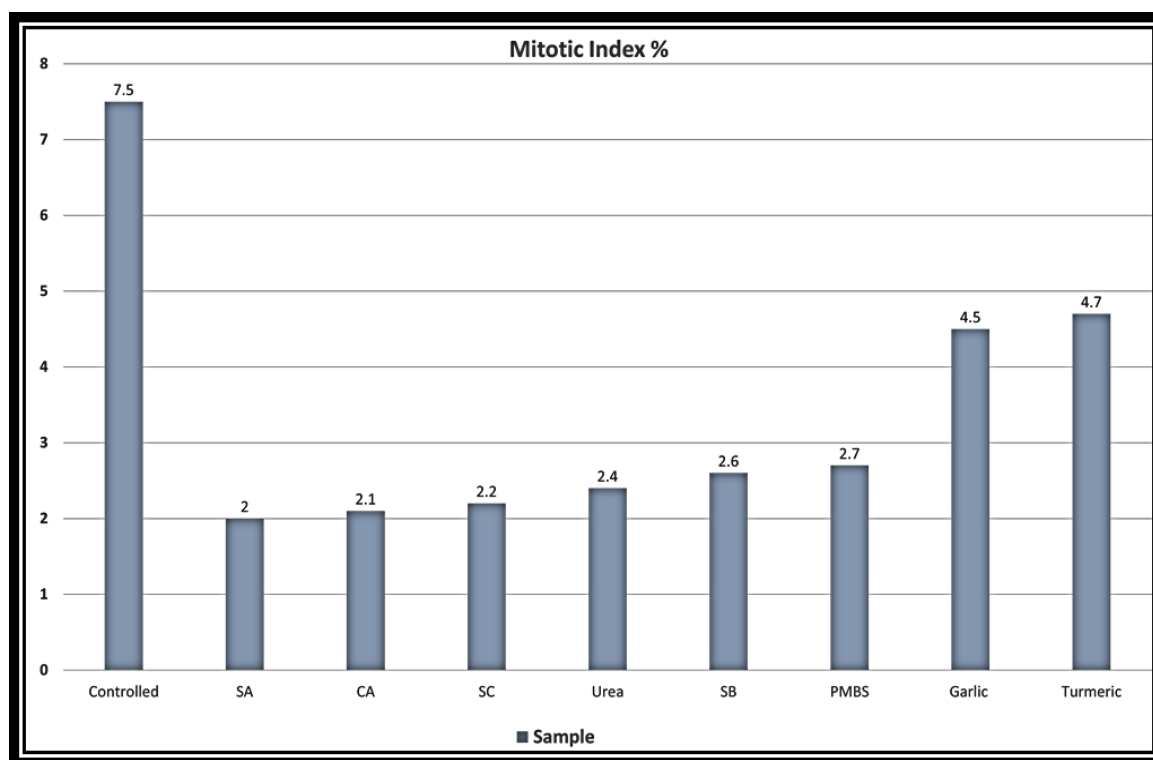


Fig. 16. Test Set: Turmeric extract – [a] normal prophase, [b] chromosomal break



Graph 1. Simple Bar Graph showing MI: Controlled – 7.5%, Mutagens: Sodium azide (SA) – 2%, Citric acid (CA) – 2.1%, Sodium citrate (SC) – 2.2%, Urea – 2.4%, Sodium benzoate (SB) – 2.6%, Potassium metabisulphite (PMBS) – 2.7%, Antimutagens: Turmeric – 4.7%, Garlic – 4.5%

Urea caused aberrations like laggards (Fig. 12), sticky chromosomes (Fig. 13) and c-mitosis (Fig. 11). Related results were detected in another researcher's experiment [6]. The mitogenic mutagenic effect in addition with the clastogenic effect of urea on the meristematic tissues of *Allium cepa* was also demonstrated by Verma et al. [16].

Sodium azide which is a known potentially harmful chemical induced chromosomal aberrations in the onion root tip cells like chromosomal break/gaps (Fig. 10) and isochromatic break. When sodium azide is dissolved in water it forms a toxic hydrogen azide gas and the production of azide ions can be the probable reason for its genotoxicity in the present test [1]. Kleinhofs and Smith (1976) disclosed that the possibility of azide mutagenesis was due to peroxide accumulation, which may also be the cause for its toxicity in this present study [17]. Sodium azide is a direct-acting mutagen that reacts directly with a DNA molecule to cause damage [18].

In the present study curcumin which is the bioactive component of turmeric and allicin which

is the bioactive component of garlic exhibited antimutagenic potential against chemical mutagen-induced chromosomal aberrations. Similar results were observed by Shukla et al (2003) against benzo(a)pyrene and dimethyl benzo(a)anthracene and cyclophosphamide-induced damage in *Salmonella* and CHO (Chinese hamster ovary) test systems [19]. Das et al (1993) evaluated the anticlastogenic activity of three doses of a crude extract of garlic (CEG) against the damage produced by mitomycin C (MMC) and cyclophosphamide (CP) in mouse bone marrow cells [20]. Shukla and Taneja (2002) analysed the antimutagenic effect of another garlic extract (GE) on newly combining it with CP [21].

The protective effect of curcumin is due to its antioxidant action, trapping of free radicals, formation of complex with mutagens, modulation of mutagen metabolism by absorbing the xenobiotics or by altering the activation and/ or detoxification of xenobiotics [22]. The modulatory role of curcumin in inhibiting mutagenicity can also be due to its antioxidant activity [23]. Sreejayan and Rao reported that the phenolic hydroxyl and methoxyl groups on the phenyl ring

and the 1,3-diketone systems are the two important structural features that contribute to antioxidant properties [24]. The probable reason for the antimutagenic effect of turmeric in this study might be because of trapping of free radicals or degeneration of harmful gas executed by its bioactive component curcumin.

Garlic also showed antimutagenic effects on the chemical-induced aberrations. Majority of the studies suggest that the most frequent mechanism related to this could be garlic's antioxidant activity [7]. In this aspect, several modes of action have been put forth considering its antigenotoxic potential. These include the effect on the metabolizer enzymes of drugs or other chemicals and preservatives, the induction of apoptosis which co-occur with an increase in the percentage of cells blocked in the G2/M phase of the cellular cycle [25].

5. CONCLUSION

Garlic and turmeric extracts effectively restricted the chromosomal aberrations caused by the chemical mutagens in the onion root tip cells. From the present test, we conclude that Garlic and Turmeric have antimutagenic and antigenotoxic properties against the chromosomal aberrations induced by chemical mutagens at specific concentrations. Since all these chemicals are used as fertilizers, food preservatives, insecticides and in medicine it is necessary to be careful while utilizing them.

CONFERENCE DISCLAIMER

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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