International Scholars Journals

African Journal of Botany ISSN: 2314-9825, Vol. 13 (1), pp. 001-010, May, 2025. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Using the Allium cepa Assay, the Aqueous Leaf Extracts of Amaranthus Spinosus Linn. have the Potential to be both Genotoxic and Antigenotoxic

Praveen Jairam and Ramesh

Universities in Malappuram, Malappuram, Kerala, India.

Accepted 30 May, 2025

Examining the potential genotoxic effects of A. spinosus leaf extracts on Allium cepa root meristematic cells as well as their antigenotoxic effects against H2O2-induced genetic damage in A. cepa was the goal of the current investigation. Following genotoxicity assay, there was a significant (P b 0.001) decrease in clastogenicity (A4, 3 h, 98.14 ± 0.70) and a significant decrease in mitotic index (MI) (A4, 3 h, 23.37 ± 1.41). Chromosome bridges, cytomixis, and an odd nucleus are among the clastogenic anomalies observed. Two H2O2 doses (7% and 3%), in antigenotoxicity experiments, caused early toxicity. The results showed a higher percentage of nuclear lesions in H2O2 treatment (99.27 ± 0.19), and a substantial decrease in this percentage following modulatory therapy (5 mg/L; 32.25 ± 7.69). This suggests that the plant extract has chemopreventive activity at a crucial concentration (5 mg/L). Since the percentage of inhibition was higher than 40% (67.51 ± 8.68), the degree of antimutagenicity at this particular concentration (5 mg/L) was substantial. The findings showed that, at a specific dosage, the aqueous plant extract of A. spinosus can prevent oxidative damage brought on by the direct-acting mutagen (H2O2).

Keywords: Allium cepa, Amaranthus spinosus, Antigenotoxicity, Genotoxicity, H2O2.

INTRODUCTION

The noxious and spinous weed Amaranthus spinosus (Family: Amaranthaceae) is utilized as a vegetable and as a tribal medicine for a variety of ailments. The herb is used in Ayurveda as a stomachic, laxative, diuretic, digestive, antipyretic, and to increase biliousness and hunger. Leprosy, bronchitis, piles, leucorrhea, and blood disorders are all cured by it (Kirtikar and Basu, 2001). Hematology is significantly impacted by the plant extract (Olufemi et al., 2003). It has anti-inflammatory (Tatiya et al., 2007), anthelmintic (Assiak et al., 2002), analgesic (Krishnamurthi et al., 2010), antidiabetic, antihyperlipidemic, spermogenic (Girija and Lakshman, 2011; Sangameswaran and Jayakar, 2008), hepatoprotective (Zeashan et al., 2009, 2008), anticancer (Cristine et al., 2013), antioxidant (Odhavo et al., 2007; Zeashan et al., 2009), and chemoprotective properties (Kumar et al., 2010).

Alkaloids, flavonoids, glycosides, phenolic acids, steroids, terpenoids, saponins, betalains, b-sitosterol, stigmasterol, rutin, catechuic tannins, and other active ingredients are present in A.spinosus. Amaranthine, isoamaranthine, hydroxycinnamates, quercetin, and kaempferol glycosides were found as betalains in the stem bark of A. spinosus (Srinivasan et al., 2005; Ibewuike et al., 1997; Rastogi and Mehrotra, 1999; Stintzing et al., 2004; Hilou et al., 2006). A coumaroyl adenosine, amaranthoside, lignan glycoside,

amaricin, stigmasterol glycoside, betaine (including glycinebetaine), and trigonelline are also present (Azhar-ul-Haq).

A key stage in the development of cancer and other degenerative processes like cardiovascular and neurological illnesses, as well as premature aging, is the induction of DNA damage, particularly oxidative damage (Finkel and Holbrook, 2000; Wiseman et al., 1995). Furthermore, a number of studies have demonstrated the potency of amaranthus extracts as scavengers of free radicals (Kumar et al., 2011; Odhavo et al., 2007). Medicinal herbs' chemical constituents can work alone or in concert to scavenge free radicals and provide antioxidant effects (Romero-Jimenez et al., 2005). Finding and characterizing the active ingredients could result in workable plans to lower the risk of cancer in people (Dearfield et al., 2002). Biologically active substances found in plants and their derivatives are utilized in traditional medicine (Aggarwal and Shishodia, 2006). Consuming a diet rich in fruits, vegetables, and other plant-based foods can lower the chance of developing chronic illnesses and cancer (Schaefer, 2002).

Singh and Dahiya (2002) investigated the antioxidant capacity of A.spinosus subjected to vehicle emissions. Analysis of the enzymes superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, and phenolic peroxidase activity revealed that A. spinosus has an excellent system for scavenging free radicals in order to fight air pollution. According to reports, betalain pigments found in Amaranthaceae plants shown strong antioxidant activity when tested using the DPPH assay. The betacyanin colors found in amaranthus leaves make them a possible substitute source of betalains. Additionally, they display action against cancer (Singh and Singh, 2011).

Therefore, the betalain content of A. spinosus extract may be the cause of its antioxidant activity (Singh et al., 1997).

The traditional practice of using medicinal plant extracts to treat human illnesses has become much more popular in recent years (Oyeyemi and Bakare, 2013). It has been demonstrated that the secondary metabolism of higher plants provides a nearly limitless supply of compounds with potential biological action (Santos et al., 2006). Antimutagens and anticarcinogens have been proposed as an efficient way to prevent human cancer and genetic illnesses in daily life (Ferguson, 1994). The ability of higher plants to modulate the activity of environmental genotoxicants is being investigated more and more. These plants are widely utilized in traditional remedies (Sreeranjini and Siril, 2011). Bioactive chemicals found in medicinal plants have the ability to prevent or reverse carcinogenesis in its early stages (Lippman et al., 1994). The goal of the current study was to use the A. cepa test system to examine the genotoxic and antigenotoxic effects of various doses of A. spinosus aqueous extract. Following treatment with H2O2, the antimutagenic investigation evaluated the ability of plant extract at lower concentrations to prevent DNA damage brought on by reactive oxygen species (ROS). Since H2O2 itself causes oxidative stress by acting as an oxidizing agent (Torbergesen and Collins, 2000), the antigenotoxicity test based on A. cepa is thought to be a simple and accessible way to screen plants for antigenotoxic potential.

2. Materials and methods

2.1. Collection of plant materials

At the Calicut University campus in Kerala's Malappuram district (latitude: 10°7′34 N; longitude: 75°53′25 E; elevation: 45–50 m), fresh plant materials of A. spinosus Linn. were gathered. Taxonomically, the plant was identified, and a voucher specimen was herbarized (CALI 123743). Certified bulbs were bought from an agricultural source for the Allium cepa assay.

2.2. Genotoxicity assay

Using a mortar and pestle, fresh aqueous leaf extracts of A. spinosus were made. One gram of leaf was weighed and crushed in one hundred milliliters of distilled water to create the stock solution. By diluting the stock solution with distilled water, the lowest concentrations of the extract—0.05 g/L (A1), 0.1 g/L (A2), 0.5 g/L (A3), and 1 g/L (A4)—were made for toxicity study. To avoid cellular changes, uniformly sized, healthy A. cepa bulbs were sorted and planted in sandy soil that had been sterilized without manure. During the peak mitotic time (9 am–10 am), germinated bulbs with good roots (1–2 cm) were gathered and cleaned in de-siccated water. Extract solutions were used to suspend the bases of onion bulbs that had roots. As controls, distilled water-treated onion bulbs were used. A few healthy root tips were removed from each bulb after treatment for varying lengths of time. These tips were then carefully cleaned with distilled water and fixed in ethanol/glacial acetic acid (2:1) fixative (modified Carnoy's solution) for one hour. Using 2% acetocarmine, mitotic squash preparations were produced using proven methods (Sharma and Sharma, 1980) following hydrolysis in 1 N HCl for 15 minutes at room temperature. To calculate the mitotic index (MI) and the proportion of chromosomal abnormalities, two slides were prepared for each treatment, and five randomly chosen spots were scored. For every treatment, the MI was computed as the proportion of cells in mitosis divided by the total number of cells counted. Additionally, the percentage of chromosomal aberrations and cytological abnormalities were scored for the cells. It was calculated by dividing the total number of cells observed by the number of abnormal cells. Between 152 and 309 cells were scanned, tabulated, and photographed using Zoom digital camera, Olympus Camedia C-4000. Photomicrographs display the most common abnormalities.

2.3. Antigenotoxicity assay

By examining H2O2-induced chromosomal abnormalities in the root tip of A. cepa while the test sample was present, the antigenotoxicity test was able to ascertain how well the test sample (A. spinosus extract) inhibited the mutagenic activity

of H2O2. Healthy onion bulbs were meticulously descaled and the old roots pulled out for this test. After that, they were set on top of tiny jars filled with distilled water and left to germinate for 48 hours at room temperature (25 ± 2 °C) in the dark. One gram of A. spinosus leaf was weighed and ground in one hundred milliliters of distilled water to create the test sample. By diluting the stock solution with distilled water, the lowest concentrations of the extract—0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L—were created for anti-genotoxicity investigation. Additionally, two H2O2 concentrations (3% and 7%) were made.

2.3.1. Treatment with H2O2

The corresponding concentrations (3% and 7%) of H2O2 were applied to the water-germinated roots for one hour. Following treatment, the roots were cut out, and cytological analyses were performed as previously mentioned. Every slide was scanned, tabulated, and photographed under a microscope. The cells were assessed for H2O2-induced cytological abnormalities.

2.3.2. Modulatory treatment with plant extracts

After a thorough wash in distilled water, the bulbs treated with H2O2 were treated for 24 hours with varying quantities of A. spinosus extract (0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L). As previously mentioned, the root tips were excised, and cytological analyses were performed. For each treatment, roughly 267–289 dividing cells from five fields were scored.
Following the modulatory therapy with plant extracts, the cells were graded for cytological abnormalities. By determining the percentage of inhibition, the chromosomal abnormalities caused by H2O2 are shown to be inhibited. The antigenotoxic potential of aqueous extract of A. spinosus was calculated using the formula:

Inhibitory activity (%) = A−B ÷ A−C × 100

A: Number of aberrant cells induced by H2O2
B: Number of aberrant cells induced by H2O2 after modulatory treat- ment
C: Number of aberrant cells induced in the negative control (distilled water)

Root tips treated with 3% and 7% H2O2 transferred to distilled water was used as positive control (PC). Root tips treated with distilled water alone are used as negative control (NC). All experiments were made in triplicate.

2.4. Statistical analysis

Data obtained from both genotoxicity and antigenotoxicity assays were subjected to statistical analysis. Duncan's multiple range test and one-way ANOVA was performed to determine mean separation and sig- nificance of treatments using SPSS version 20, SPSS Inc., Chicago, USA.

3. Results

3.1. Genotoxicity assay

The outcomes show that A. spinosus has both genotoxic and antigenotoxic effects on the root tip cells of A. cepa. When root tips were exposed to greater concentrations (A1, A2, A3, and A4) of the test solution, acute toxicity was seen in the genotoxic testing. When compared to the control (96.99 ± 1.37), a significant decrease in the MI was seen mostly at higher concentrations. After three hours of therapy, MI reached its peak concentration (A4) at 23.37 ± 1.41, and after one hour, it reached its lowest concentration (A1) at 69.47 ± 5.83 (Table 1). When compared to therapy, the control group showed much less mitotic inhibition. The MI of the treatment and control groups differed significantly (p b 0.001) (Table 1). The decrease in MI indicates that the leaf extract from A. spinosus has antimitotic and antiproliferative properties.
When the plant extract was applied to the root tips of A. cepa, a genotoxicity experiment showed several clastogenic abnormalities (Figs. 1–3). It was discovered that the ab- normality percentage depended on concentration. In comparison to the control [0.00 ± 0.00 (½ h) and 0.79 ± 0.79 (2 h)], the greatest anomaly was noted at the highest concentration of plant extract [A4, 95.09 ± 2.66 (1 h) and 98.14 ± 0.70 (3 h)] (Table 1). Nuclear lesions, erosions, strange mononucleate, binucleate, and trinucleate cells, chromosome bridges, cytomixis, giant cells, enucleated cells, strap cells and strap nuclei, hyperchromasia, nuclear breakage and disintegration, nuclear dim-inution, nuclear emergence, nuclear budding, nuclear peaks, chained chromosomes, coagulated chromosomes, pulverized chromosomes, chromosome gaps, etc. were among the clastogenic abnormalities seen (Figs. 1–3). A significant (p b 0.001) number of clastogenic abnormalities were produced by the plant extract.

3.2. Antigenotoxicity assay

The most common abnormality caused by H2O2 is found to be nuclear lesions (Fig. 4; both 3% and 7%). When compared to nuclear lesions, other abnormalities such as sticky chromosomes, nuclear erosions, disrupted chromosomes, etc., are less common. At a critical concentration (5 mg/L), the A. spinosus extract was shown to significantly (p b 0.001) decrease the H2O2-induced nuclear lesions in modulatory treatment. Since the proportion of inhibition was less than 25%, all other examined conditions were deemed to be unimportant. Compared to positive controls [99.27 ± 0.19 for 3% H2O2 and 99.76 ± 0.11 for 7% H2O2] and other concentrations of plant extract [10 mg/L, 3% H2O2, 88.41 ± 5.80; 0.5 mg/L, 3% H2O2, 90.16 ± 2.72], the percentage of lesions seen at this effective concentration was lower (32.25 ± 7.69) (Table 2).

Compared to PC, which had the largest incidence of lesions, negative controls had the lowest percentage (0.76 ± 0.38). By determining the percentage of inhibition, the chromosomal abnormalities caused by H2O2 are shown to

have inhibitory activity. At this critical dosage (5 mg/L) of plant extract, the percentage of nuclear lesions inhibited was 67.51 ± 8.68 (Table 2). It was discovered that this nuclear lesion inhibition was substantial. Since the proportion of inhibition was higher than 40%, the degree of antimutagenicity at this particular concentration was substantial. The findings showed that, at a specific concentration, the aqueous plant extract of A. spinosus can prevent the direct-acting mutagen (H2O2).

4. Discussion

Because anti-mutagenic chemicals may also be anticarcinogens, antimutagenicity screening was justified. Though numerous chemicals can contribute to the carcinogenic process without causing mutations, this should be remembered. By changing the methylation and acetylation of DNA and histones, these substances can change gap junctional intercellular communication, trigger intracellular signaling, and change patterns of gene expression. Instead than via mutation, they might cause cancer through a "epigenetic mechanism" (Trosko and Upham, 2005). Therefore, while antimutagenicity screening is undoubtedly crucial, it does not address all pertinent factors in the battle against cancer (Verschaeve & Van Staden, 2008).
Similar to earlier research where extracts of various medicinal plants reduced mitotic cell division in A. cepa root tips, all plant extract concentrations tested in the genotoxicity assay had mitodepressive effects on cell division (Frescura et al., 2013; Khanna and Sharma, 2013; Lamsal et al., 2010). The reduction of MI suggests that the aqueous leaf extract of A. spinosus suppresses mitotic activity in A. cepa. The components in the aqueous extracts that have cytotoxic effects are the cause of the decrease in MI, as MI is a quantitative assessment of the mitotic activities in an organism or specific organ (Sreeranjini and Siril, 2011). In all living things, MI is a valid indicator of cytotoxicity (Smaka-Kinel et al., 1996). The reduced rate of MI can be used to calculate the degree of cytotoxicity.
While a decrease below 50% typically has sublethal consequences (Panda and Sahu, 1985) and is referred to as the cytotoxic limit value (Sharma, 1983), a reduction below 22% in relation to negative controls might have a lethal effect on the organism (Antonsiewicz, 1990). The reduction in MI explains the possible cytotoxicity and points to the studied extracts' antiproliferative, mitodepressive, and inhibitory activities on the meristematic cells at the root tip of A. cepa.

A decrease in mitotic activity may result from stopping the cell cycle's G2 phase, which would prevent the cell from going through mitosis, or from suppression of DNA synthesis (Sudhakar et al., 2001). According to Majewska et al. (2003), it may also result from decreased ATP levels and compromised nucleoprotein synthesis, which supply energy for chromosomal movement, microtubule dynamics, and spindle elongation. Because A. spinosus leaf extract inhibits cell reproduction, its remarkably low MI values may be utilized in anticancer research.

Numerous clastogenic abnormalities were brought up by the genotoxicity experiment. There are two types of chromosomal aberrations: structural and numerical. The latter arises from chromosomal material breaking or exchanging (Khanna and Sharma, 2013; Timothy et al., 2014). Cytotoxicants' direct effect on the chromosomes caused the clastogenic aberrations. According to Muralimov et al. (2013), cytomixis (Fig. 1d, e) is the movement of nuclei from one plant cell to another via a particular kind of intercellular channel called a cytomictic channel, which is different from plasmodesmata in terms of size and shape. The notion that cytomixis was a general reaction to a direct mechanical or chemical influence on the plant cells was experimentally disproved by Baquar and Husain (1969).

Furthermore, it has been shown that cytomixis is totally stopped by any direct harmful effect on the cell (Liu et al., 2007). When a plant is subjected to stressors such mutagens, elevated temperatures, alterations in the ploidy level, hybridization, and so forth, the rate of cytomixis typically rises (Kravets, 2011; Sidorchuk et al., 2007). During cytomixis, migrating nuclei typically divide to generate micronuclei (Mursalimov et al., 2013). In order to directly connect the nuclei of neighboring cells via cytomictic channels, a nuclear bridge (Fig. 1e) is also created during cytomixis (Mursalimov and Deineko, 2011). Micronuclei, nuclear bridges, binucleate cells (Fig. 1a, c, n), and enucleated cells (Fig. 1f) are the most common outcomes of such migrations (Mursalimov et al., 2013) (Negron-Ortiz, 2007). Binucleate cells also arise when the cytokinesis phase of cell division is inhibited (Khanna and Sharma, 2013).
The extract's activity resulted in enucleated (ghost) cells (Fig. 1f), which had cytoplasmic and nuclear damage that killed cells. A dead cell that has a discernible outline but an unstainable nucleus and cytoplasm is known as a "ghost cell." Apoptosis, often known as cell death, is a biological process that occurs in living things. The demise of cells was brought on by elevated levels of harmful substances (Çelik and Aslantürk, 2010). The effectiveness of cancer-treating drugs is frequently correlated with changes in cell and nuclear size (Fig. 1g, o, t; Fig. 2i). Kang et al. (2011) revealed that when arctigenin, a natural plant lignin, was applied to SW 480 human colon cancer cells, nuclear expansion was seen. Increased chromosomal contraction and condensation, or possibly depolymerization of DNA and partial dissolution of nucleoproteins, are the causes of chromosome stickiness (Fig. 2k; Fig. 3h, i). Stickiness of the chromosomes indicates toxic effects, which are typically irreversible and most likely cause cell death. Chromatin bridges may occur during the transfer of uneven chromatid exchange and result in structural chromosome mutations (Fig. 1c; Fig. 2l; Fig. 3b, c, e, g, h, j, l, n, o) (Khanna and Sharma, 2013).
The presence of specific phytochemical components in the extracts may be the cause of the observed clastogenicity elicited in A. cepa root meristematic cells.

At higher concentrations, certain naturally occurring plant compounds, including flavanols, polyphenols, alkaloids, and tannins, have been linked to chromosomal damage (Ene

and Osuala, 1990; Hayakawa et al., 1999; Perez-Carreon et al., 2002; Yamanaka et al., 1997), and at lower concentrations, the same extracts can act as antimutagens. When considering plant polyphenols as therapeutic agents, it is important to identify their opposing action at varying extract concentrations (Perez-Carreon et al., 2002). This serves as a warning against the careless application of these botanicals in conventional therapy. Nonetheless, anticancer research can benefit from the active phytochemicals that produced clastogenicity.

Because of its sensitivity and strong correlation with mammalian test systems in vitro (i.e., using bone marrow cells of Wistar rats; El-Shabbaby et al., 2003; Teixeira et al., 2003; Ventura et al., 2008), the A. cepa root chromosomal aberration assay has been described as an effective test system and is frequently used to assess the genotoxic potential of chemicals in the environment. One of the numerous benefits of the Allium test as a genotoxicity screening method is that A. cepa root cells have a mixed function oxidase system that can activate genotoxic substances or promutagens. Furthermore, this test is a crucial in vivo experiment in which the roots are exposed to the compounds of interest directly, potentially causing DNA damage that might be linked to human DNA. Animals and plants have physically similar chromosomes, and they seem to react to mutagen therapy similarly to mammals and other eukaryotes (Nefic et al., 2013).

By decreasing and reversing cytological abnormalities, particularly the nuclear lesions brought on by H2O2 (3%), the plant extract demonstrated chemopreventive activity in the current investigation. The cells experienced oxidative stress as a result of being exposed to H2O2, which may be why treatment with H2O2 caused a significant induction of nuclear lesions. Mercykutty and Stephen (1980) observed that A. cepa root meristematic cells had nuclear lesions generated by plant-derived compounds. This could be because cytotoxicants disintegrated a portion of the nuclear material. After being treated with Corymbia leaf extracts, the root tip meristem of A. cepa showed a profound occurrence of nuclear lesions, which indicates the total loss of nuclei (Saj and Thoppil, 2006). The organization of the cytoskeleton is known to be impacted by active oxygen (O) forms that arise during oxidative stress (Egorova et al., 2001). It is well known that ROS can lead to cellular damage by oxidizing biomolecules. ROS can induce tissue damage, which can include oxidation of vital enzymes and damage to proteins and DNA. Numerous diseases linked to free radicals may subsequently arise as a result of these occurrences. Numerous endogenous defensive and protective systems, including different enzymes and non-enzymatic antioxidants, regularly fight the harmful effects of ROS in the human body. Additionally, antioxidant chemicals found in foods, cosmetics, and herbal medicines may help these self-defense mechanisms (Kähkönen et al., 1999).

While lower quantities of plant extract (0.5 mg/L, 1 mg/L, 5 mg/L, and 10 mg/L) used for antigenotoxicity assays were not found to be genotoxic, higher concentrations of plant extract (A1, A2, A3, and A4) utilized for genotoxicity testing caused significant cytogenetic damages in A. cepa root meristematic cells. One of the lesser concentrations (5 mg/L) utilized in the antigenotoxicity experiment was shown to be successful in reversing the genotoxicity (nuclear lesions) caused by H2O2 (3%). Therefore, it can be said that this concentration of A. spinosus (5 mg/L) is essential for reversing the genotoxicity caused by H2O2. Because the same extract or sample can have both mutagenic and antimutagenic qualities, studies of this type are therefore highly complex. The same plant extracts must be examined for potential mutagenic and antimutagenic reactions. "Janus carcinogens and mutagens" refers to a sample that exhibits both mutagenicity and antimutagenicity (Zeiger, 2003). An extract may occasionally be antimutagenic at greater concentrations and mutagenic at lower concentrations, or vice versa. Instead of testing the test chemical at a single concentration, a range of values should be used to handle this problem. Ferguson (2001) claims that there is proof that some compounds have the ability to both cause and stop DNA damage. It is known that complex combinations of phytochemicals that can function antagonistically, additively, or synergistically make up crude extracts. Reduced amounts of the components causing the observed aberrations during H2O2 treatment may be the cause of the decreased frequency of aberrations in modulatory treatment, or it may be the direct outcome of specific antioxidant chemicals found in the extract. It's possible that certain plants have compounds that can alter the genotoxicity of other compounds. According to earlier research, the antioxidant activity of A. spinosus is caused by the presence of betalain, a water-soluble pigment that contains nitrogen (Guaadaoui et al., 2014; Stintzing and Carle, 2004). Enzymes, cell membranes, and genetic material will all suffer damage and dysfunction as a result of free radical assaults on proteins, lipids, and DNA during H2O2 treatment (Droge, 2002; Fang et al., 2002). It is clear from this viewpoint that there is growing interest in the advantageous features of betalains that help human defense mechanisms. A recent study examined the structure–activity connections of different betacyanins and betaxanthins (two components of betalain) from Amaranthaceae members in relation to their ability to scavenge free radicals (Cai et al., 2003). It was discovered that the structural characteristics of the corresponding betalains were connected to the antioxidant potential. More hydroxy and imino residues in betaxanthins enhanced their ability to scavenge free radicals. Glycosylation decreased the antioxidant capability of betacyanins, but acylation often increased it. Additionally, compared to 6-O-glycosylated betacyanins, 5-O-glycosylated structures generated poorer antioxidant values. The purportedly encouraging physiological and pharmacological effects of betalains on humans that have been reported recently will need to be determined by future research (Kanner et al., 2001; Wettasinghe et al., 2002).

According to the current study, the extract's active ingredients may have scavenged the free radicals produced by H2O2 and lessened the genotoxic damage. Additionally, the substances might improve DNA synthesis or repair, or they might even stop some chemicals from bioactivating (Kuroda et al., 1992). The test system's

frequency of nuclear lesions decreased after receiving A. spinosus extract treatment, suggesting that the likelihood of carcinogenesis may be decreased. According to earlier research, the total phenolic content of plant extracts may be responsible for their antigenotoxic potential (Maurich et al., 2004). These can combine with metal ions to produce potent ligand complexes (Ferguson, 2001; Hale et al., 2001; Rice-Evans et al., 1997). Two additional explanations for this protection could be that the plant extracts adsorb the mutagens similarly to how pyrrole pigments like hemin and chlorophyllin have been linked to carcinogen adsorption (Ferguson et al., 2004; Ikuma et al., 2006); or the extracts could trigger DNA glicosylase enzymes, which can repair DNA by alkylating DNA bases (Steele and Kelloff, 2005).

When assessing a sample's antimutagenic potential, a value less than 25% inhibition of the mutagen activity denotes a weak or non-antimutagenic effect; a value between 25% and 40% denotes a moderate effect; and a value greater than 40% denotes strong antimutagenicity (Verschaeve and Van Staden, 2008). With the exception of 5 mg/L, none of the other doses show any protection against H2O2-induced DNA damage.

Lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methio-9, tyrosine, and tryptophan are among the amino acids found in A. spinosus (Tee et al., 1987). According to De Toranzo et al. (1983), tyrosine and methionine have a protective effect against acute liver damage caused by carbon tetrachloride. According to Kumar et al. (2010), the methanolic extract of A. spinosus leaves contains amino acids, flavonoids, ter-penoids, saponins, glycosides, and phenolic compounds that have hepatoprotective and antioxidant properties against acute liver injury in rats caused by paracetamol. The genotoxic and antigenotoxic potential of A. spinosus extracts appears to be caused by the presence of these bioactive chemicals.

5. Conclusion

The current investigation, which verified A. spinosus's mutagenic and anti-mutagenic qualities, undoubtedly merits more consideration. The active ingredients found in this species were identified by earlier research. Because there are insufficient in vivo and in vitro research that could describe the mechanism of this plant's anticancer activity, its effectiveness has not yet been scientifically verified. In addition to the plant's purported medicinal benefits, the mutagenic and antimutagenic qualities offer additional health benefits. A. spinosus aqueous leaf extract may also be turned into antiproliferative, chemopreventive, or anticarcinogenic medicines, according to the study's findings. Lastly, keep in mind that a lot of anticancer medications are mutagens. Therefore, it is not always the case that an antimutagen should be given in addition to the chemotherapeutic drug, as is occasionally recommended. It is true that lowering the mutagenicity would also lower the drug's clinical efficacy (Verschaeve and Van Staden, 2008).

6. Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

Author kindly acknowledges Kerala State Council for Science, Tech- nology & Environment for providing financial assistance through KSCSTE fellowship.

References

Aggarwal, B.B., Shishodia, S., 2006. Molecular targets of dietary agents for prevention and therapy of cancer. Biochemical Pharmacology 71, 1397–1421.

Antonsiewicz, D., 1990. Analysis of the cell cycle in the root meristem of Allium cepa under the influence of ledakrin. Folia Histochemica Et Cytobiologica 26, 79–96.

Assiak, I.E., Olufemi, B.E., Ayonde, G.O., Onigemo, M.A., 2002. Preliminary studies on the effects of Amaranthus spinosus leaf extract as an anthelmintic in growing pigs. Trop- ical Veterinarian 20, 126–129.

Azhar-ul-Haq, M., Afza, N., Khan, S.B., Muhammad, P., 2006. Coumaroyl adenosine and lig- nan glycoside from Amaranthus spinosus Linn. Polish Journal of Chemistry 80, 259–263.

Baquar, S.R., Husain, S.A., 1969. Cytoplasmic channels and chromatin migration in the meiocytes of Arnebia hispidissima (Sieb.) DC. Annals of Botany 33, 821–837.

Blunden, G., Yang, M., Janicsak, M.I., Carabot-Cuervo, A., 1999. Betaine distribution in the Amaranthaceae. Biochemical Systematics and Ecology 27, 87–92.

Cai, Y., Sun, M., Corke, H., 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of Agricultural and Food Chemistry 51, 2288–2294.

Çelik, T.A., Aslantürk, Ö.S., 2010. Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with Allium test. Journal of Biomedicine and Biotechnology 2010, 1–8.

Cristine, D.O., Melinda, L., Ade, Z., Ajeng, D., Anas, S., Rizky, A., 2013. Anticancer properties of daily-consumed vegetables Amaranthus spinosus, Ipomoea aquatica, Apium graveolens and Manihot utilisima to LNCaP prostate cancer cell lines. Journal of Natural Pharmaceuticals 4, 67–70.

De Toranzo, E.G., De Ferreyra, E.C., De Fenos, O.M., Castro, J.A., 1983. Prevention of carbon tetrachloride-induced liver necrosis by several amino acids. British Journal of Exper- imental Pathology 64, 166–171.

Dearfield, K.L., Cimino, M.C., Mc Carroll, N.E., Mauer, I., Valcovic, L.R., 2002. Genotoxicity risk assessment: a proposed classification strategy. Mutation Research 521, 121–135. Droge, W., 2002. Free radicals in the physiological control of cell function. Physiological Reviews 82, 47–95.

Egorova, A.B., Uspenskaya, Y.A., Mithukkina, S.V., Stravikkskaya, E.Y., 2001. Damage of cy- toskeleton and cell membrane by apoptosis. Advances in Current Biology 121, 502–510.

El-Shabbaby, O.A., Migid, A.H.M., Soliman, M.I., Mashaly, I.A., 2003. Genotoxicity screening of industrial waste water using the Allium cepa chromosome aberration assay. Pakistan Journal of Biological Sciences 6, 23–28.

Ene, E.E., Osuala, C.L., 1990. The mutagenic potentials of water extract of Borreria filiformis (Hiern) Hatch and Dalz. and Vinca rosea Linn. Nigerian Journal of Botany 3, 35–40.
Fang, Y.Z., Yang, S., Wu, G., 2002. Free radicals, antioxidants and nutrition. Nutrition 18, 872–879.
Ferguson, L.R., 1994. Antimutagens as cancer chemopreventive agents in the diet. Muta- tion Research 307, 395–410.
Ferguson, L.R., 2001. Role of plant polyphenols in genomic stability. Mutation Research 475, 89–111.
Ferguson, R.L., Philpott, M., Karunasinghe, N., 2004. Dietary cancer and prevention using antimutagens. Toxicology 198, 147–159.
Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.
Frescura, V.D., Kuhn, A.W., Laughinghouse, I.V.H.D., Nicoloso, F.T., Lopes, S.J., Tedesco, S.B., 2013. Evaluation of the allelopathic, genotoxic, and antiproliferative effect of the me- dicinal species Psychotria brachypoda and Psychotria birotula (Rubiaceae) on the ger- mination and cell division of Eruca sativa (Brassicaceae). Caryologia 66, 138–144.
Girija, K., Lakshman, K., 2011. Anti-hyperlipidemic activity of methanol extracts of three plants of Amaranthus in triton-WR 1339 induced hyperlipidemic rats. Asian Pacific Journal of Tropical Biomedicine 1, 62–65.
Guaadaoui, A., Saddik, R., Bouali, A., Boukhatem, N., Benchat, N.E., Hamal, A., 2014. Betalains, polyacetylenes and tocols as biocompounactives: a concise review for enriching the bioactivity concept. International Journal of Nutrition and Food Sci- ences 3, 230–237.
Hale, K.L., Mc Grath, S.P., Lombi, E., Stack, S.M., Terry, N., Pickering, I.J., George, G.N., Pilon- Smits, E.A., 2001. Molybdenum sequestration in Brassica species. A role for anthocy- anins. Plant Physiology 126, 1391–1402.
Hayakawa, F., Kimura, T., Hoshimo, N., Ando, T., 1999. DNA cleavage activities of (–)-epi- gallocatechin, (–)-epicatechin, (+)-catechin, and (–)-epigallocatechin gallate with various kind of metal ions. Bioscience Biotechnology and Biochemistry 63, 1654–1656.
Hilou, A., Nacoulma, O.G., Guiguemde, T.R., 2006. In vivo antimalarial activities of extracts from Amaranthus spinosus L. and Boerhaavia erecta L. in mice. Journal of Ethnopharmacology 103, 236–240.
Ibewuike, J.C., Ogundaini, A.O., Bohlin, L., Ogungbamila, F.O., 1997. Antiinflammatory ac- tivity of selected Nigerian medicinal plants. Nigerian Journal of Natural Products and Medicine 1, 10–14.
Ikuma, N.E.M., Passoni, H.M., Biso, I.F., Longo, C.M., Cardoso, P.R.C., Campaner, S.L., Varanda, A.E., 2006. Investigation of genotoxic and antigenotoxic activities of Melampodium divaricatum in Salmonella typhimurium. Toxicology in Vitro 20, 361–366.
Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47, 3954–3962.
Kang, K., Lee, H.J., Yoo, J.H., Jho, E.H., Kim, C.Y., Kim, M., Nho, C.W., 2011. Cell and nuclear enlargement of SW 480 cells induced by a plant lignan, arctigenin: evaluation of cel- lular DNA content using fluorescence microscopy and flow cytometry. DNA and Cell Biology 30, 623–629.
Kanner, J., Harel, S., Granit, R., 2001. Betalains—a new class of dietary cationized antioxi- dants. Journal of Agricultural and Food Chemistry 49, 5178–5185.
Kapadia, G., Balasubramanian, V., Tokuda, H., Iwashima, A., Nishino, H., 1995. Inhibition of 12-O-tetradecanoylphorbol-13-acetate induced Epstein- Barr virus early antigen acti- vation by natural colorant. Cancer Letters 115, 173–178.
Kapadia, G., Tokuda, H., Harukuni, K., Takao, M., Nishino, H., 1996. Chemoprevention of lung and skin cancer by Beta vulgaris (beet) root extract. Cancer Letters 100, 211–214.
Khanna, N., Sharma, S., 2013. Allium cepa root chromosomal aberration assay: a review. Indian Journal of Pharmaceutical and Biological Research 1, 105–119.
Kirtikar, K.R., Basu, B.D., 2001. Indian medicinal plants. Oriental Enterprises second ed. New Connaught Place, Dehradun, Uttranchal, India, pp. 2832–2836.
Kravets, E., 2011. The role of cell selection for pollen grain fertility after treatment of barley sprouts (Hordeum distichum L.) with UV-B irradiation. Acta Biologica Slovenica 54, 31–41.
Krishnamurthi, S.K.M., Venugopalan, R., Suresh, K.S., Perumal, P., 2010. Evaluation of anal- gesic activity of Amaranthus spinosus Linn. leaves in mice. Journal of Pharmacy Re- search 3, 3088–3089.
Kumar, B.S.A., Lakshman, K., Jayaveera, K.N., Shekar, D.S., Nandeesh, R., Velmurugan, C., 2010. Chemoprotective and antioxidant activities of methanolic extract of Amaranthus spinosus leaves on paracetamol induced-liver damage in rats. Acta Medica Saliniana 39, 68–74.
Kumar, B.S.A., Lakshman, K., Nandeesh, R., Kumar, P.A.A., Manoj, B., Kumar, V., Shekar, D.S., 2011. In vitro alpha-amylase inhibition and in vivo antioxidant potential of Amaranthus spinosus in alloxan-induced oxidative stress in diabetic rats. Saudi Journal of biological sciences 18, 1–5.
Kuroda, Y., Jain, A.K., Tezuka, H., Kada, T., 1992. Antimutagenicity in cultured mammalian cells. Mutation Research 267, 201–209.
Lamsal, K., Ghimire, B.K., Sharma, P., Ghimiray, A.K., Kim, S.W., Yu, C.Y., Chung, I.M., Lee, Y.S., Kim, J., Shakya, S.R., 2010. Genotoxicity evaluation of the insecticide ethion in root of Allium cepa L. African Journal of Biotechnology 9, 4204–4210.
Lippman, S.M., Benner, S.E., Hong, W.K., 1994. Cancer chemoprevention. Journal of Clinical Oncology 12, 851–873.
Liu, H., Guo, G.Q., He, Y.K., Lu, Y.P., Zheng, G.C., 2007. Visualization on intercellular move- ment of chromatin in intact living anthers of transgenic tobacco expressing histone 2B-CFP fusion protein. Caryologia 60, 1–20.
Majewska, A., Wolska, E., Śliwińska, E., Furmanowa, M., Urbańska, N., Pietrosiuk, A., Zobel, A., Kuras, M., 2003. Antimitotic effect, G2/M accumulation, chromosomal and ultra- structure changes in meristematic cells of Allium cepa L. root tips treated with the extract from Rhadiola roots. Caryologia 56, 337–351.

Maurich, T., Pistelli, L., Turchi, G., 2004. Anti-clastogenic activity of two structurally related pterocarpans purified from Bituminaria bituminosa in cultured human lymphocytes. Mutation Research 561, 75–81.
Mercykutty, V.C., Stephen, J., 1980. Adriamycin induced genetic toxicity as demonstrated by Allium test. Cytologia 45, 769–777.
Mursalimov, S.R., Deineko, E.V., 2011. An ultra structural study of cytomixis in tobacco pollen mother cells. Protoplasma 248, 717–724.
Mursalimov, S.R., Sidorchuk, Y.V., Deineko, E.V., 2013. New insights into cytomixis: specif- ic cellular features and prevalence in higher plants. Planta 238, 415–423.
Nefic, H., Musanovic, J., Metovic, A., Kurteshi, K., 2013. Chromosomal and nuclear alter- ations in root tip cells of Allium cepa L. induced by alprazolam. Medical Archives 67, 388–392.
Negron-ortiz, V., 2007. Chromosome numbers, nuclear DNA content, and polyploidy in consolea (Cactaceae), an endemic cactus of the Caribbean Islands. American Journal of Botany 94, 1360–1370.
Odhavo, B., Beekrum, S., Akula, U.S., Baijnath, H., 2007. Preliminary assessment of nutri- tional value of traditional leafy vegetables in Kwazulu-Natal. South Africa, Journal of Food Composition and Analysis 20, 430–435.
Olufemi, B.E., Assiak, I.E., Ayoade, G.O., Onigemo, M.A., 2003. Studies on the effects of Amaranthus spinosus leaf extract on the hematology of growing pigs. African Journal of Biomedical Research 6, 149–150.
Oyeyemi, I.T., Bakare, A.A., 2013. Genotoxic and anti-genotoxic effect of aqueous extracts of Spondias mombin L., Nymphea lotus L. and Luffa cylindrica L. on Allium cepa root tip cells. Caryologia 66, 360–367.
Panda, B.B., Sahu, U.K., 1985. Induction of abnormal spindle function and cytokinesis inhi- bition in mitotic cells of Allium cepa by the organophosphorus insecticide fensulfothion. Cytobios 42, 147–155.
Patkai, G., Barta, J., Varsanyi, I., 1997. Decomposition of anticarcinogen factors of the beet root during juice and nectar production. Cancer Letters 114, 105–106.
Perez-Carreon, J.I., Cruz-Jimenez, G., Licea-Vega, J.A., Popoca, E.A., Fazenda, S.F., Villa- Trevino, S., 2002. Genotoxic and anti-genotoxic propertice of Calendula officinalis ex- tract in rat liver cell cultures treated with diethylnitrosamine. Toxicology In Vitro 16, 253–258.
Rastogi, R.P., Mehrotra, B.N., 1999. Compendium of Indian Medicinal Plants. Lucknow. Rice-Evans, C.A., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science 2, 152–159.
Romero-Jimenez, M., Sanchez, J.C., Analla, M., Serrano, A.M., Moraga, A.A., 2005. Genotoxicity and antigenotoxicity of some traditional medicinal herbs. Mutation Re- search 585, 147–155.
Saj, O.P., Thoppil, J.E., 2006. Cytotoxic studies and exploration of essential oil in two spe- cies of Corymbia. Journal of Cytology and Genetics 7, 131–139.
Sangameswaran, B., Jayakar, B., 2008. Anti-diabetic, anti-hyperlipidemic and spermato- genic effects of Amaranthus spinosus Linn. on streptozotocin-induced diabetic rats. Journal of Natural Medicines 62, 79–82.
Santos, F.V., Mesquita, S.F.P., Faria, M.J.S.S., Poersh, A., Maciel, M.A.M., Pinto, A.C., Morimoto, H.K., Cólus, I.M.S., 2006. Absence of mutagenicity in somatic and germ cells of mice submitted to subchronic treatment with an extract of Croton cajucara Benth. (Euphorbiaceae). Genetics and Molecular Biology 29, 159–165.
Schaefer, E.J., 2002. Lipoprotein, nutrition and heart disease. The American Journal of Clin- ical Nutrition 75, 191–212.
Sharma, C.B.S.R., 1983. Plant meristems as monitors of genetic toxicity of environmental chemicals. Current Science 52, 1000–1002.
Sharma, A.K., Sharma, A., 1980. Chromosome Techniques—Theory and Practice. third ed. Butterworths, London.
Sidorchuk, Y.V., Deineko, E.V., Shumny, V.K., 2007. Peculiarities of cytomixis in pollen mother cells of transgenic tobacco plants (Nicotiana tabacum L.) with mutant pheno- type. Cell and Tissue Biology 1, 570–576.
Singh, A.B., Dahiya, P., 2002. Antigenic and allergenic properties of Amaranthus spinosus pollen. A common growing weed in India. Annals of Agricultural and Environmental Medicine 9, 147–151.
Singh, N., Singh, P., 2011. Amaranth: potential source for flour enrichment. In: Victor, R.P., Ronald, R.W., Vinood, P. (Eds.), Flour and Breads and Their Fortification in Health and Disease Prevention. Academic Press, Elsevier, London, Burlington, San Diego, pp. 101–111.
Singh, B.P., Malhotra, M., Sridhara, S., Gaur, S.N., 1997. Relevance of birth month in respi- ratory allergy. Allergy 52, 232–233.
Smaka-Kinel, V., Stegnar, P., Lovaka, M., Toman, J., 1996. The evaluation of waste, surface and ground water quality using the Allium test procedure. Mutation Research 368, 171–179.
Sreeranjini, S., Siril, E.A., 2011. Evaluation of anti-genotoxicity of the leaf extracts of Morinda citrifolia Linn. Plant, Soil and Environment 57, 222–227.
Srinivasan, K., Kaul, C.L., Ramarao, P., 2005. Partial protective effect of rutin on multiple low dose streptozotocin-induced diabetes in mice. Indian Journal of Pharmacology 37, 327–328.
Steele, V.E., Kelloff, G.J., 2005. Development of cancer chemopreventive drugs based on mechanistic approaches. Mutation Research 591, 16–23.
Stintzing, F.C., Carle, R., 2004. Functional properties of anthocyanins and betalains in plants, food and in human nutrition. Trends in Food Science and Technology 15, 19–38.
Stintzing, F.C., Kammerer, D., Schieber, A., Hilou, A., Nacoulma, O., Carle, R., 2004. Betacyanins and phenolic compounds from Amaranthus spinosus L. and Boerhaavia erecta. Zeitschrift für Naturforschung. Section C 59, 1–8.
Sudhakar, R., Gowda, K.N.N., Venu, G., 2001. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of Allium cepa. Cytologia 66, 235–239.
Tatiya, A.U., Surana, S.J., Khope, S.D., Gokhale, S.B., Sutar, M.P., 2007. Phytochemical inves- tigation and immunomodulatory activity of Amaranthus spinosus Linn. Indian Journal of Pharmaceutical Education and Research 44, 337–341.

Tee, L.B.G., Davies, D.S., Seddon, C.E., Boobis, A.R., 1987. Species differences in the hepato- toxicity of paracetamol are due to differences in the rate of conversion to its cytotoxic metabolite. Biochemical Pharmacology 36, 1041–1052.
Teixeira, R.O., Camparoto, M.L., Mantovani, M.S., Vicentini, V.E.P., 2003. Assessment of two medicinal plants Psidium guajava L. and Achillea millefollium L. in in vitro and in vivo assays. Genetics and Molecular Biology 26, 551–555.
Timothy, O., Idu, M., Olorunfemi, D.I., Ovuakporie-Uvo, O., 2014. Cytotoxic and genotoxic properties of leaf extract of Icacina trichantha Oliv. South African Journal of Botany 91, 71–74.
Torbergesen, A.C., Collins, A.R., 2000. Recovery of human lymphocytes from oxidative DNA damage, the apparent enhancement of DNA repair by carotenoids is probably simply an antioxidant effect. European Journal of Nutrition 39, 80–85.
Trosko, J.E., Upham, B.L., 2005. The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. Mutagenesis 20, 81–92.
Ventura, B.C., Angelis, D.F., Marin-Morales, M.A., 2008. Mutagenic and genotoxic effects of the Atrazine herbicide in Oreochromis niloticus (Perciformes, Cichlidae) detected by the micronuclei test and the comet assay. Pesticide Biochemistry and Physiology 90, 42–51.
Verschaeve, L., Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. Journal of Ethnopharmacology 119, 575–587.
Wettasinghe, M., Bolling, B., Plhak, L., Parkin, K., 2002. Screening for phase II enzyme- inducing and antioxidant activities of common vegetables. Journal of Food Science 67, 2583–2588.
Wiseman, H., Kaur, H., Halliwell, B., 1995. DNA damage and cancer: measurement and mechanism. Cancer Letters 93, 113–120.
Yamanaka, N., Oda, O., Nagao, S., 1997. Green tea catechins such as (–)-epicatechin and (–)-epigallocatechin accelerate Cu2+ induced low density lipoprotein oxidation in propagation phase. FEBS Letters 401, 230–234.
Zeashan, H., Amresh, G., Singh, S., Rao, C.V., 2008. Hepatoprotective activity of Amaranthus spinosus in experimental animals. Food and Chemical Toxicology 46, 3417–3421.
Zeashan, H., Amresh, G., Singh, S., Rao, C.V., 2009. Hepatoprotective and antioxidant activity of Amaranthus spinosus against CCl4 induced toxicity. Journal of Ethnopharmacology 125, 364–366.
Zeiger, E., 2003. Illusions of safety: antimutagens can be mutagens, and anticarcinogens can be carcinogens. Mutation Research 543, 191–194.

Table 1
Mitotic index and abnormality percentage in control and various treatments.

Treatment	Time (h)	Total cells ± SE	Dividing cells ± SE	No. of clastogenic cells ± SE	Mitotic index (%) ± SE	Clastogenicity (%) ± SE
Control	½	245 ± 4.05^{b}	238 ± 5.50^{c}	0.00 ± 0.00^{a}	96.99 ± 1.37^{c}	0.00 ± 0.00^{a}
	1	201 ± 9.35^{a}	183 ± 5.56^{c}	2 ± 1.20^{a}	91.12 ± 3.48^{c}	1.12 ± 0.56^{a}
	2	169 ± 16.17^{a}	150 ± 11.53^{b}	1 ± 1.33^{a}	88.91 ± 2.36^{d}	0.79 ± 0.79^{a}
	3	163 ± 2.84^{a}	146 ± 7.21^{c,d}	0.6 ± 0.66^{a}	89.30 ± 2.90^{d}	0.42 ± 0.42^{a}
A_1	½	152 ± 16.04^{a}	88 ± 16.04^{a}	108 ± 21.45^{b}	57.56 ± 6.22^{b}	71.12 ± 10.98^{b}
	1	220 ± 19.37^{a,b}	151 ± 9.56^{b}	155 ± 24.26^{b}	69.47 ± 5.83^{b}	69.38 ± 4.91^{b}
	2	258 ± 27.71^{b}	143 ± 23.33^{b}	240 ± 21.88^{b}	55.24 ± 4.59^{c}	93.53 ± 2.30^{c,d}
	3	250 ± 23.44^{b}	125 ± 21.38^{b,c}	222 ± 14.0^{b}	51.05 ± 10.76^{b,c}	89.16 ± 2.66^{b}
A_2	½	244 ± 14.88^{b}	144 ± 13.29^{b}	213 ± 16.38^{c}	59.18 ± 5.18^{b}	87.06 ± 2.17^{c}
	1	270 ± 13.69^{b,c}	119 ± 15.19^{a}	244 ± 15.67^{c}	43.81 ± 3.78^{a}	90.37 ± 1.24^{c}
	2	268 ± 13.13^{b}	102 ± 3.17^{a}	223 ± 16.12^{b}	38.33 ± 2.67^{b}	83.56 ± 6.87^{b,c}
	3	278 ± 9.87^{b,c}	104 ± 10.17^{a,b}	254 ± 12.05^{c}	37.48 ± 3.52^{a,b}	91.17 ± 3.21^{b,c}
A_3	½	284 ± 5.17^{c}	114 ± 6.88^{a,b}	274 ± 5.66^{d}	40.30 ± 3.02^{a}	96.47 ± 0.26^{c}
	1	260 ± 18.77^{b,c}	108 ± 6.96^{a}	246 ± 18.47^{c}	42.05 ± 4.17^{a}	94.58 ± 0.68^{c}
	2	299 ± 2.40^{b}	190 ± 6.35^{c}	246 ± 2.30^{b}	63.69 ± 2.10^{c}	82.18 ± 0.88^{b}
	3	268 ± 3.46^{b,c}	177 ± 6.80^{d}	234 ± 5.29^{b,c}	66.10 ± 3.22^{c}	87.37 ± 2.87^{b}
A_4	½	303 ± 3.21^{c}	107 ± 5.50^{a}	293 ± 2.72^{d}	35.32 ± 1.92^{a}	96.92 ± 0.57^{c}
	1	304 ± 17.0^{c}	99 ± 2.08^{a}	288 ± 9.93^{c}	32.79 ± 2.12^{a}	95.09 ± 2.66^{c}
	2	309 ± 4.04^{b}	79 ± 8.35^{a}	303 ± 3.51^{c}	25.68 ± 2.73^{a}	98.06 ± 0.74^{d}
	3	305 ± 13.64^{c}	71 ± 3.17^{a}	300 ± 28.04^{d}	23.37 ± 1.41^{a}	98.14 ± 0.70^{c}

Control (distilled water); A_1, A_2, A_3 and A_4—*A. spinosus* aqueous leaf extract concentrations 0.05 g/L, 0.1 g/L, 0.5 g/L and 1 g/L, respectively; SE, standard error. Means within a column followed by the same letters are not significantly different at *P* b 0.001 as determined by Duncan′s multiple range tests.

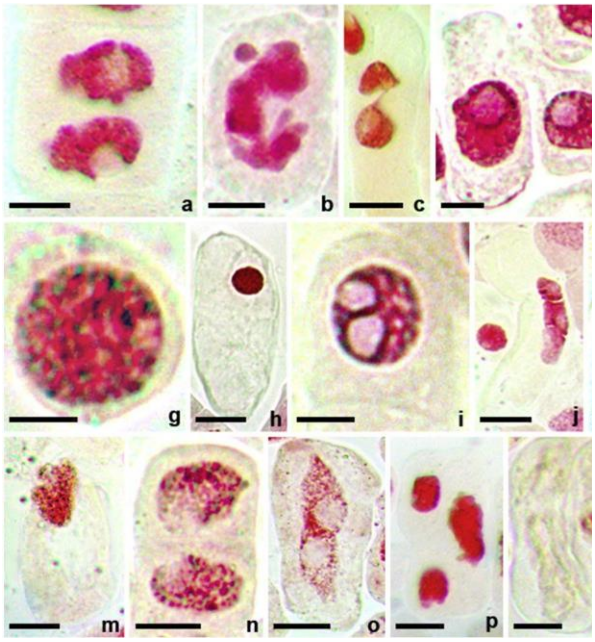

Fig. 1. (a) Bizarre binucleate cell; (b) bizarre nucleus; (c) bridged binucleate cell; (d) cytomixis and nuclear budding; (e) nuclear bridge at cytomixis; (f) enucleated cell; (g) giant nucleus;
(h) hyperchromasia; (i) multiple nuclear lesions; (j) nuclear breakage; (k) nuclear budding, lesion and erosion; (l) nuclear diminution; (m) nuclear emergence; (n) nuclear erosion in a binucleate cell; (o) giant cell showing nuclear lesions and erosion; (p) bizarre trinucleate cell; (q) two cells showing stages of nuclear disintegration; (r) cytomixis; (s) nuclear peaks; (t) giant strap cells. Bar, 20 µm. Clastogenic aberrations induced by *A. spinosus* leaf extract on *A. cepa* root tip cells.