Ind. J. Pharm. Edu. Res., 2025; 59(3s):s1012-s1019.
https://www.ijper.org

Original Article

Antigenotoxic Activity of *Carissa spinarum* (L.) Leaves Extract against Allethrin-Induced Genotoxicity in *Allium cepa* (L.) Meristematic Cells

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ABSTRACT

Aim: The purpose of this research was to evaluate the phytochemical composition and antigenotoxic properties of *Carissa spinarum* leaf extract against allethrin-induced genotoxicity in *Allium cepa* (L.) meristematic cells. **Materials and Methods:** Phytochemical screening methods were employed to analyze and quantify the phenolic, flavonoid and terpenoid content, as well as to conduct histochemical assessments on the extract to understand the efficacy and its antigenotoxic mechanism. **Results:** The phytochemical analysis of *C. spinarum* leaves revealed the presence of tannin, saponins, flavonoids, steroids, terpenoids, anthraquinones, polyphenols and glycosides, with the absence of alkaloids and anthocyanin in the aqueous extract. Significant amounts of flavonoids, terpenoids and total phenols were detected in *C. spinarum* leaves. Histochemical analysis further confirmed the presence of these phytochemicals. The *A. cepa* L. root tip model shows that *C. spinarum* extract enhances root growth and reduces genotoxicity in a dose-dependent manner. Root length increases significantly at 0.4 mg/mL and genotoxicity decreases with extract concentrations (79.86%, 50.39% and 5.49%) compared to Allethrin-treated group II (96.31%). **Conclusion:** The genotoxicity percentage increased in the Allethrin-treated group, indicating genotoxicity, while it decreased in the *C. spinarum* extract-treated group, confirming its antigenotoxic effect, as reported in our study.

Keywords: Antigenotoxic, *Carissa spinarum*, Genotoxicity, Histochemical, Phytochemical.

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Received: 07-02-2025;
Revised: 27-03-2025;
Accepted: 16-06-2025.

INTRODUCTION

Genotoxicity, within the realm of genetics, pertains to the existence of substances that exert detrimental effects on the genetic components of cells, such as DNA and RNA, thereby jeopardizing the overall integrity of the cell. Genotoxins, which are capable of inducing genotoxicity, act as mutagens and can instigate damage to DNA or chromosomal material, ultimately resulting in mutations. These genotoxic agents may include both chemical substances and radiation. Genetic toxicology, a specialized scientific discipline, is dedicated to investigating agents or substances that can adversely affect the DNA and chromosomes of cells. It is crucial to differentiate genotoxicity from mutagenicity, as while all mutagens are inherently genotoxic, not all genotoxic substances necessarily lead to mutagenic outcomes.[1]

Genotoxicity studies encompass a range of *in vitro* and *in vivo* tests specifically designed to detect substances or compounds that damage genetic material, either directly or indirectly, through various mechanisms. These are essential for identifying hazards related to DNA damage and its stabilization.[2] Genetic changes play a role in the intricate processes of hereditary effects and malignancy, involving DNA damage fixation through gene mutations, large-scale chromosomal damage, recombination, or numerical chromosomal changes. These tests are pivotal in determining whether a compound has the potential to induce genotoxicity and carcinogenicity when they yield positive results.[3] Regulatory bodies globally mandate the inclusion of information on the genotoxic potential of new drugs as an essential component of the safety evaluation process. Typically, the assessment of genotoxicity is carried out in conjunction with other toxicological endpoints as part of comprehensive safety evaluations.[4]

Increased focus is currently directed towards medicinal plants owing to their potential to provide a myriad of advantages to society, especially within the realms of medicine and pharmacology. The therapeutic effectiveness of these plants is attributed to their phytochemical constituents, which exert distinct

DOI: 10.5530/ijper.20255649

pharmacological effects on the human body. Phytochemicals are chemical compounds produced through metabolic reactions occurring during plant growth. These compounds play a crucial role in the medicinal attributes of plants and their applications in healthcare and pharmaceuticals.[5] *C. spinarum* is a thorny, evergreen shrub found in tropical and subtropical regions. It contains various phytochemicals such as flavonoids, alkaloids, saponins, tannins and phenolic compounds, which contribute to its medicinal properties, including antioxidant, antimicrobial and anticancer activities.[6,7]

The objective of this study was to explore the antigenotoxic potential of *C. spinarum* leaves against genotoxicity induced by allethrin in *A. cepa* meristematic cells. Additionally, the research aimed to identify and analyze the bioactive compounds present in *C. spinarum* leaves. The focus was on understanding how these leaves could counteract the genotoxic effects caused by allethrin, ultimately contributing valuable insights into the protective properties of *C. spinarum* in the context of DNA mutations and cancer risk.

MATERIALS AND METHODS

Collection of plant and Preparation of extract

The *C. spinarum* leaves were collected during January 2023 from Vallam, Thanjavur, Tamil Nadu, India. These leaves underwent multiple washes with distilled water to eliminate impurities. Subsequently, the leaves were ground using a grinder mixture. For the aqueous extract, distilled water was used and for the ethanol extract, a 70:30 ethanol-to-water ratio was employed.

To prepare the extracts, 20 g of the powdered leaves were mixed with 200 mL of distilled water and boiled in a covered beaker for 5 min. The mixture was then cooled to room temperature for 10 min and filtered through Whatman filter paper to remove any particulate matter. The stock solution was diluted with distilled water to obtain the desired concentrations. Fresh extracts were prepared daily before each experiment to ensure maximum efficacy.

Phytochemical screening

The chemical tests on the extract were conducted following standard procedures to identify its constituents, as outlined in established protocols.[8-10] Total phenols were quantified using the method described by Edeoga *et al*., (2005), while flavonoids were determined following the procedure outlined by Bohm and Kocipai-Abyazan (1994). The estimation of total terpenoid content was carried out based on the method established by Ferguson (1956).

Furthermore, histochemical tests were conducted following the procedures outlined by Paul JP (2014). These methodologies were specifically chosen to analyze and quantify the phenolic, flavonoid and terpenoid content in the *Carissa spinarum* extracts.

For phenolic content analysis, the extracts were subjected to a ferric chloride test. A portion of the extract was mixed with a few drops of Ferric Chloride solution ($FeCl_3$) in a test tube. The development of a blue or green color indicated the presence of phenolic compounds.

Flavonoid content was assessed using the Aluminum Chloride ($AlCl_3$) test. A portion of the extract was mixed with $AlCl_3$ solution in a test tube. The formation of a yellow color confirmed the presence of flavonoids. Terpenoid content was evaluated using the Salkowski test. The extract was mixed with concentrated sulfuric acid in a test tube. The appearance of a reddish-brown color in the lower layer indicated the presence of terpenoids. Histochemical evaluations on the extract involved microscopic analysis of tissue sections stained with specific reagents to visualize the distribution and localization of these chemical constituents within the plant tissues.[11-14]

Evaluation of antigenotoxic activity using *A. cepa*

Small bulbs (1.5-2.0 cm in diameter) of *A. cepa* were acquired from a local market. To prepare the bulbs for testing, the outer layers and the dry basal plates were removed, taking care not to damage the root primordia. 6 bulbs were used for each extract sample and were 1st placed in distilled water (pH 7.3) for 48 hr to promote root growth. After this initial growth period, the roots were treated with *C. spinarum* leaf extracts at concentrations of 0.1 mg/mL, 0.2 mg/mL and 0.4 mg/mL. Root tips that had newly formed were periodically cut and checked for any visible morphological changes. Only bulbs with root lengths between 2 and 2.5 cm were selected for further study; those with significantly shorter or longer roots were excluded.[15]

The antigenotoxic activity was evaluated using *A. cepa* roots involved preparing fresh onion bulbs by cleaning and promoting root growth in distilled water. The bulbs were divided into 5 groups, including controls treated with distilled water only and others exposed to allethrin alone or allethrin combined with *C. spinarum* extract at concentrations of 0.1 mg/mL, 0.2 mg/mL and 0.4 mg/mL. After treatment, root growth was measured and roots were harvested for cytological analysis. Chromosomal aberrations were identified in root tips stained with a DNA-specific stain and examined under a microscope. The percentage of genotoxicity was calculated based on aberrations observed, comparing treated groups to controls. For each concentration of the extract, 5 bulbs were used. Tap water (pH 7.3) served as the negative control, while Allethrin was used as the positive control due to its known mutagenic properties, which cause DNA damage through oxidative stress, which leads to the generation of Reactive Oxygen Species (ROS). These ROS can cause various forms of DNA damage, including single and double-strand breaks, base modifications and cross-linking of DNA strands.[16]

After 24 hr of exposure to the test substances, root tips were collected from the bulbs, fixed in a 3:1 (v/v) ethanol: glacial acetic

acid solution and stored overnight at 4ºC. The next day, the fixed root tips were transferred to 70% (v/v) aqueous ethanol and refrigerated until use. For each bulb, 5 slides were prepared using 5 root tips, which were hydrolyzed in 1 N Hydrochloric acid (HCl) for 3 min. The root tips were then squashed on microscope slides and stained with 2% (w/v) acetic-orcein. These slides were examined under a microscope and the data is presented as the mean±SE of experiments with triplicate dose.[17]

Effect of *C. spinarum* on DNA fragmentation in *A. cepa*

The percentage of DNA fragmentation was calculated in *A. cepa* roots treated with *C. spinarum* extract using the gel electrophoresis method; root tips are first collected and homogenized in a lysis buffer to extract DNA. The DNA is then purified using standard phenol-chloroform extraction and ethanol precipitation techniques. Isolated DNA samples are loaded onto an agarose gel and subjected to electrophoresis, which separates the DNA fragments based on size. After electrophoresis, the gel is stained with a DNA-specific dye such as ethidium bromide and visualized under UV light. This method provides a quantifiable measure of DNA fragmentation, enabling assessment of genotoxic effects and protective capabilities of treatments. The percentage of DNA fragmentation is calculated by:

$$\% \text{ DNA Fragmentation} = \left(\frac{\text{Tail DNA intensity}}{\text{Total DNA intensity (tail+head)}}\right) \times 100$$

The following parameters were used for determination of cytotoxicity and genotoxicity:

(i) The Mitotic Index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{total number of cells observed}} \times -100$$

and (ii) chromatin aberrations were used as endpoints for determination of genotoxicity effects:

$$\% \text{ Genotoxicity} = \left(\frac{\text{Number of aberrant cells}}{\text{Total number of cells or root tips}}\right) \times 100$$

RESULTS

Phytochemicals are natural compounds that are found in various plant sources and these metabolic compounds encompass a wide range of chemical classes, such as alkaloids, flavonoids, coumarins, tannins, terpenes, terpenoids, phenols, gums, polysaccharides and glycosides and contribute to the diverse health-promoting properties of plant-based foods and medicinal plants.[10] The current study conducted on *C. spinarum* extract has unveiled the presence of biologically active constituents. The phytochemical analysis of *C. spinarum* leaves is summarized in Table 1.

The results of the phytochemical screening of *C. spinarum* extract reveal the presence of tannins, saponins, flavonoids, steroids, terpenoids, anthraquinones, polyphenols and glycosides in the aqueous extract. However, alkaloids and anthocyanin were not detected. In contrast, the ethanol extract of *C. spinarum* shows the presence of tannins, saponins, flavonoids, steroids, terpenoids, anthraquinones, polyphenols, glycosides and alkaloids, with the absence of anthocyanin.

Quantitative analysis

The quantitative analysis of *C. spinarum* extract indicated the presence of flavonoids, terpenoids and phenols. The results showed a substantial content of terpenoids (10 mg/g), flavonoids (50 mg/g) and phenols (362.10 mg/g) as presented in Table 2. These phytoconstituents were quantified using standard methods.

Histochemical analysis

In this investigation, *C. spinarum* extract underwent treatment with specific chemicals and reagents. Treatment of the *C. spinarum* extract with diluted ammonia and H_2SO_4 resulted in a yellow

Table 1: Qualitative analysis of Phytochemicals in *C. spinarum* extract.

Sl. No.	Phytochemicals	Aqueous extract	Ethanol extract
1	Tannin	+	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	++	++
6	Alkaloids	-	+
7	Anthraquinone	+	++
8	Polyphenol	+	++
9	Glycoside	++	++
10	Anthocyanin	-	-

(+) Indicates Presence; (++) Moderately present.

coloration, signifying the presence of flavonoids. Furthermore, when the *C. spinarum* extract was subjected to a few drops of $FeCl_3$, it underwent a color change to black, confirming the presence of tannins.[18] These color changes served as indicators of the presence of these specific phytochemical compounds in the *C. spinarum* extract.

The *C. spinarum* extract, when treated with toluidine blue, exhibited a blue-green/red coloration, indicating the presence of polyphenols. Similarly, when the *C. spinarum* extract was treated with a few drops of dinitrophenol hydrazine, it turned orange, suggesting the presence of terpenoids. These observations, as recorded in Table 3 and Plate 1, provided additional confirmation of the presence of specific phytochemical compounds in the plant material under study.

Antigenotoxic activity of *C. spinarum*

The present study also investigated the protective effect of *C. spinarum* extract and its various concentrations on the genotoxicity caused by Allethrin at 0.5 ppm concentration (EC_{50} value). This testing method is both rapid and cost-effective, enabling the exploration of universal mechanisms applicable to meristematic plant cells and facilitating the extrapolation of findings to animal cells.[19]

Plate 2 illustrates the morphometric analysis of *A. cepa* roots following treatment with *C. spinarum* extract. The water control group showcases normal growth with extended root length. Conversely, group II treated with Allethrin exhibits a reduction in root length compared to control group I. However, when subjected to different concentrations (0.1, 0.2 and 0.4 mg/mL) of *C. spinarum* extract, there is a gradual increase in root growth, demonstrating a dose-dependent response.

Notably, the highest dose (0.4 mg/mL) significantly enhances root length compared to the other doses (Plate 3). The percentage of genotoxicity decreases with increasing concentrations of *C. spinarum* extract (79.86%, 50.39% and 5.49%) as compared to group II (96.31%) in Table 4. These findings suggest the potential protective effects of *C. spinarum* extract against genotoxicity and its positive impact on root growth.[20]

The assessment of genotoxic activity was carried out using the meristematic cells of *A. cepa* root, which have been widely employed in screening drugs with antigenotoxic properties. These plant roots have distinct regions, one of which is the area of cell division located just beyond the root cap, extending a few millimeters thereafter. Cells within this region undergo frequent divisions, resulting in a higher rate of cell division compared to other root tissues.[21] The mitotic index, an indicator of cell division, was calculated for various treatment groups and is presented in Table 5. The results demonstrate that the mitotic index was significantly reduced in the Allethrin-only group (23.50%) compared to the control (88.07%), indicating a genotoxic effect. However, treatment with *Carissa spinarum* extract at various concentrations (100 µg/mL, 200 µg/mL and 400 µg/mL) showed a dose-dependent recovery in the mitotic index, with the highest concentration restoring it to 88.58%. These findings indicate the antigenotoxic potential of *Carissa spinarum* extract in counteracting Allethrin-induced genotoxic effects.

The division of meristematic cells in plants shares similarities with the cell division observed in human cancer cells. Consequently, these meristematic plant cells can serve as a valuable tool for the preliminary screening of drugs with potential anticancer activity. Although questions may arise about the extrapolation of results from plant tissue to animals and, ultimately, to humans, it's noteworthy that Khilman observed plant cells to be more resistant

Table 2: Quantitative phytochemical analysis of *C. spinarum* extract.

Sl. No.	Phytochemicals	Results (mg/g)
1	Terpenoids	10.00
2	Flavonoids	50.00
3	Phenol	362 .10

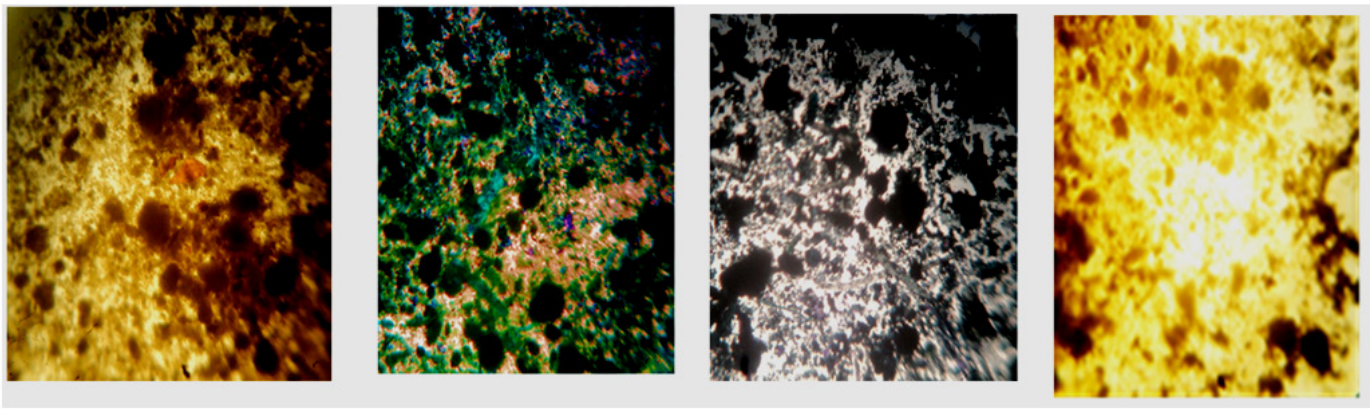

Plate 1: Histochemical analysis of *C. spinarum* extract.

to colchicine, a potent anticarcinogen that inhibits microtubule formation. This observation suggests that chemicals influencing plant chromosomes may also impact animals.[22] Therefore, the Allium assay stands as a rapid, highly sensitive and reproducible bioassay for detecting genotoxicity.

In the present study, an increase in DNA fragmentation (as observed in Table 6) in group II indicated the genotoxic effect of Allethrin. Conversely, when treated with *C. spinarum*, a decrease in DNA fragmentation was observed, indicating the antigenotoxic effect. These findings suggest that *C. spinarum* has the potential to counteract the genotoxic effects induced by Allethrin, preventing the fragmentation of DNA strands and potentially protecting cells from genetic damage.

DISCUSSION

Mutations cause various cellular defects, impacting morbidity and mortality in living organisms. Many natural and synthetic substances are mutagenic or carcinogenic. However, plants and their products can have anti-mutagenic, anti-genotoxic and anti-carcinogenic effects. This study examines the genotoxic effect of allethrin and the antigenotoxic effect of *C. spinarum* extract using the *Allium cepa* assay. This assay is effective for assessing the genotoxicity of environmental pollutants and the antigenotoxicity of medicinal plants. It is relevant for *in vivo* studies, as plant and animal chromosomes share similar morphology and responses to toxicants. Similar study was performed by many researchers and observed antigenotoxic effect of plant extracts against the toxicity of various toxicants or mutagens Hassain *et al*., (2011) conducted a screening of phytochemical constituents from the methanol leaf extract of Bombax malabaricum.[23] Thamizh Mozhi *et al*., (2011) subjected various organic solvent extracts of Pedalium murex to preliminary phytochemical screenings.[24] Additionally, a study selected 53 traditionally used medicinal plants from the western region of India to analyze their qualitative phytochemical content, total phenol and flavonoid contents. Pascaline *et al*., (2011) conducted a screening of phytochemical constituents from some medicinal plants used by the Nandis of South Nandi District, Kenya.[25]

In a study conducted by Kumar *et al*., (2013), preliminary phytochemical screening of *Lasia spinosa* (Lour) Thwaites leaves was investigated.[26] The screening revealed that both methanol and aqueous extracts contained alkaloids and carbohydrates. Phenolic compounds were present in all solvent extracts except the petroleum ether extract. Glycosides were found in chloroform, ethyl acetate and aqueous extracts, while saponins were present in methanol and aqueous extracts. Flavonoids were detected solely in the ethyl acetate extract.

Leo Stanley *et al*., (2011) reported the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids in the leaves of *C. pedata*.[27] Dinesh Kumar *et al*., (2011) similarly identified that terpenoids, flavonoids and tannins are present in C. trifolia.[28] In a study by Rajmohanan *et al*., (2014), a preliminary phytochemical analysis of various leaf extracts of *C. pedata* revealed the presence of carbohydrates, flavonoids, tannins, phenolic compounds and terpenes. These findings offer valuable insights into the phytochemical composition of this plant species.[29]

In the current study, antigenotoxic effects were observed at all concentrations of the test extract, as evidenced by macroscopic parameters such as a reduction in root length. These findings indicate inhibition of root growth. The study also examined the mitotic index at various extract concentrations, revealing the extract's efficiency in inhibiting the growth of cancer cells, potentially by affecting microtubules. This could involve either disrupting existing microtubules or promoting the formation of *C. spinarum* microtubules, preventing them from breaking down. Consequently, the cells become congested with microtubules, hindering their ability to continue growing and dividing.

Table 3: Histochemical analysis of *Carissa spinarum* extract.

Phytochemical	Result
Terpenoids	+
Polyphenol	++
Flavonoids	++
Tannin	++

(+) represents presence and (++) represents high concentrations.

Table 4: Effect of *C. spinarum* extract on root length and root number of *A. cepa*.

Groups	Root Number and length (cm)				% of Genotoxicity
	Before treatment		After treatment		
	Number	length (cm)	length (cm)	% of Root growth	
Group I (Control)	18	1.74	2.54	31.49	--
Group II (Allethrin only)	12	2.55	2.58	1.16	96.31
Group III (Allethrin+100 µg/mL)	14	3.10	3.34	6.34	79.89
Group IV (Allethrin+200 µg/mL)	16	0.81	0.96	15.62	50.39
Group V (Allethrin+400 µg/mL)	22	1.51	2.15	29.76	5.49

Table 5: Effect of *C. spinarum* leaves on the mitotic index of *A. cepa*.

Groups	Dividing cells	Non-dividing cells	Total cells	Mitotic index (%)
Group I (Control)	110	15	125	88.07
Group II (Allethrin only)	21	68	89	23.50
Group III (Allethrin+100 µg/mL)	51	54	105	48.26
Group IV (Allethrin+200 µg/mL)	61	33	94	65.06
Group V (Allethrin+400 µg/mL)	98	12	111	88.58

Table 6: Effect of *C. spinarum* on DNA fragmentation in *A. cepa*.

Parameters	Group I	Group II	Group III	Group IV	Control
DNA (%) Fragmentation	3.61	13.07	6.99	4.84	4.17

Moreover, phytosterols present in the extract were found to influence apoptosis, a process critical to regulating tumor growth. Tumor growth is influenced by a balance between cell proliferation and apoptosis rates. Apoptosis, or programmed cell death, can be influenced by phytosterols. The study suggests that the observed effects on apoptosis, along with the impact on microtubules, contribute to the antigenotoxic properties of the *C. spinarum* extract, potentially making it a promising candidate for further investigation in cancer research.[30]

The study results demonstrated the excellent antigenotoxic activity of *C. spinarum* extract, leading to a significant increase in non-dividing cells. Consequently, cells arrested in mitosis and eventually underwent apoptosis, a programmed cell death. Similar outcomes have been reported in studies conducted.[17,31-33] These findings suggest that the genotoxic activity of *C. spinarum* might be attributed to the presence of triterpenoids and phenolic compounds in the extracts.

These results provide support for the traditional therapeutic use of this plant as a genotoxic agent in the Indian system of medicine, validating its potential effectiveness in preventing genetic damage and supporting its potential application in the field of healthcare and medicine. In this study, the active phytochemicals present in *C. spinarum* might have scavenged the free radicals generated by allethrin, reducing genotoxicity levels. These phytochemicals may also enhance the DNA synthesis or repair system. Treatment with *C. spinarum* extract/fractions reduced the frequency of chromosomal aberrations in *Allium cepa*, indicating its anti-carcinogenic nature. The antimutagenic or antigenotoxic potential of plant extracts is often attributed to their phenolic content. *C. spinarum* contains various bioactive compounds, including C-glycosides, flavonoids, terpenoids, tannins, steroids, saponins, alkaloids, glycosides and anthraquinone. These compounds are likely responsible for the antigenotoxic potential of the extracts/fractions of *C. spinarum*.

Plate 2: Antigenotoxic activity of *C. spinarum* extract in experimental set-up.

The results observed strongly suggest the antigenotoxic effect of *C. spinarum*. Genetic material damage is likely to occur as a result of interactions between genotoxic substances found in the plant and the structure and sequence of DNA. These substances are likely to interact at specific locations or base sequences within the DNA structure, causing disjunction that can result in damage and mutations.[31] This mechanism has been validated by researchers and is commonly utilized to evaluate the genoprotective potential of medicinal plants. *C. spinarum*, through its manifestation of antigenotoxic properties, showcases its capability to safeguard genetic material from the detrimental effects of genotoxic substances. This underscores its potential significance in the realms of natural medicine and genetic protection research.[34-36]

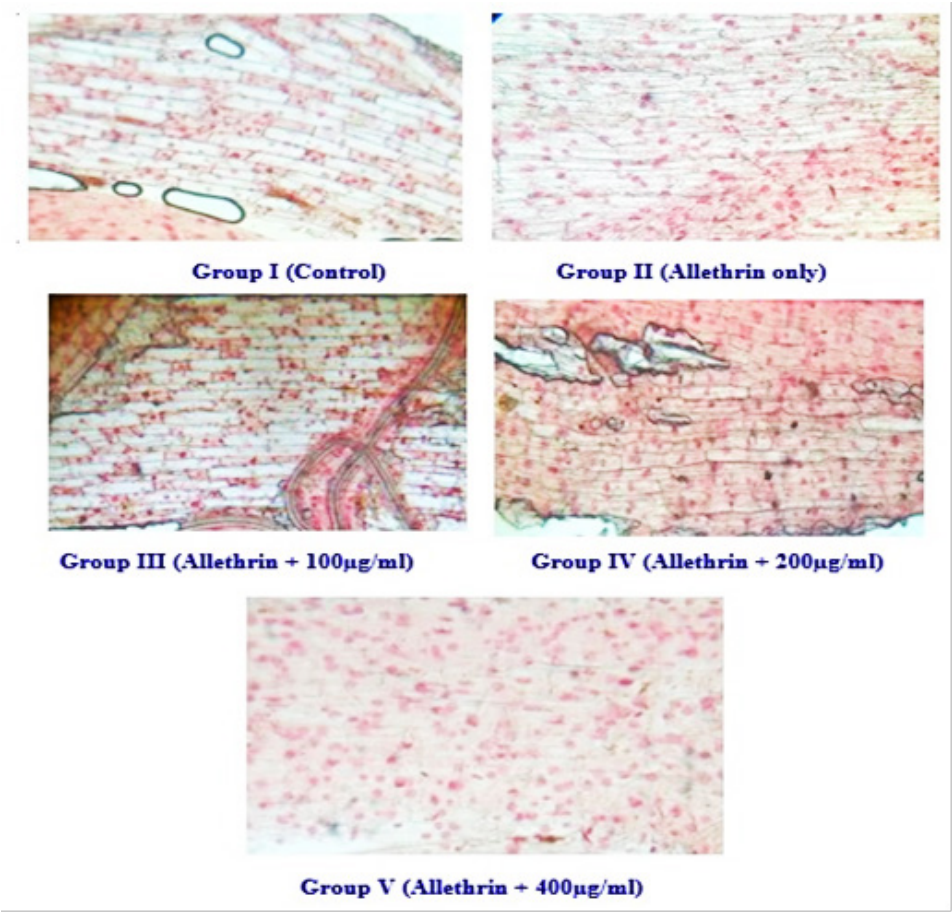

Plate 3: Effect of *C. spinarum* extract on the mitotic index of *A. cepa*.

CONCLUSION

In conclusion, the present study confirms the antigenotoxic property of *C. spinarum*, thereby enhancing the medicinal value of the plant's claimed therapeutic benefits. The study suggests that the fractions of *C. spinarum* extract have the potential to be developed into anticarcinogenic agents, emphasizing the need for further anti-genotoxicological investigations for human welfare. The study also indicates that the extract of *C. spinarum* is rich in phytochemicals, particularly alkaloids and saponins, which contribute to its remarkable antigenotoxic properties. However, further research in animal systems is warranted to explore other potential anti-genotoxic effects of *C. spinarum*. Continued studies in this area can provide valuable insights into the potential therapeutic applications and benefits of *C. spinarum* in protecting genetic material and preventing genetic damage.

ACKNOWLEDGEMENT

The author wishes to thank Periyar Maniammai University for providing the necessary facilities and support to carry out this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DNA: Deoxyribonucleic acid; **RNA:** Ribonucleic acid; **ROS:** Reactive oxygen species; **HCl:** Hydrochloric acid; **MI:** Mitotic index.

SUMMARY

This study aimed to evaluate the phytochemical composition and antigenotoxic properties of *C. spinarum* leaf extract against genotoxicity induced by allethrin in *A. cepa* (L) meristematic cells. Phytochemical screening methods were employed to analyze phenolic, flavonoid and terpenoid content and histochemical assessments were conducted to understand the extract's efficacy and antigenotoxic mechanism. Significant amounts of flavonoids, terpenoids and total phenols were detected. Genotoxicity increased in the allethrin-treated group but decreased in the *C. spinarum* extract-treated group, confirming its antigenotoxic effect. This research suggests the potential of *C. spinarum* leaf extract as a natural remedy against allethrin-induced Genotoxicity.

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Cite this article: Varadharajan S, Pandian P. Antigenotoxic Activity of *Carissa spinarum* (L.) Leaves Extract against Allethrin-Induced Genotoxicity in *Allium cepa* (L.) Meristematic Cells. Indian J of Pharmaceutical Education and Research. 2025;59(3s):s1012-s1019.