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**ORIGINAL ARTICLE**

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# A Comprehensive Comparative Study on *Cascabela thevetia* (L.) Lippold, Seed Aqueous Extract-mediated Escalation of Abiotic Stress and Cellular Genotoxicity: Insights from Multivariate Allelochemical Analysis vis-a-vis Employment of Plant Bioassays (*Lathyrus sativus* L., and *Allium sativum* L., germinating root tip cells).

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**Background***: Cascabela thevetia* (L.) Lippold, a popular member of family Apocynaceae, (Yellow Oleander), being one of the preferred weapons for suicides in villages of India is grown in garden and roadside ways as a tree a. Pharmacologically active constituents include terpenoids, fla-vonoid, steroids and glycosides in seeds.

**Purpose**: To identify the different alkaloids present in the seeds of *Cascabela thevetia* (L.) Lippold, and to evaluate the extent of severity of the toxic components (alkaloids) eubequitiously omnipotent in the aqueous decoction in different eukaryotic genomes. The other purpose lies in identifying this plant seed's potency as a ready source of active but varied levels of phytochemicals that could be exploited as future "novel bioactives" as therapeutic leads in drug discovery.

**Results**: Some important alkaloids viz., Hordenine, Ismine, Trisphaeridine, Crinine, Galanthamine, Anhydrolycorine, Assoanine, Galanthine, Incartine, Lycorine and Galwesine, are being identified in the dried seed powder of *Cascabela thevetia (L.) Lippold.* In addition to the presence of Carbohydrates, proteins, tannins, phenolics, terpenoids and alkaloids in the dried seed powder. The aqueous extract was found *to* produce significant root length inhibition in pretreated germinating *Lathyrus sativus* L. seeds with serially diluted concentrations (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively). There was significant occurrence of abnormal cells in the aforementioned doses of pre-treated seeds in *Lathyrus* sativus L., and *Allium sativum* L., root tip cells. There was significant induction of almost all types of clastogenic and aneugenic chromosomal aberrations. The said pre-treatments induced significant increase in nuclear budding, double nucleus, nuclear bridges, micronuclei followed by karyorrhexis and karyolysis. There was high frequency of occurrence of giant cells and apoptotic cells. At 40 mg/ml pre-treatment root tip cells there were higher degree of apoptotic responses showing nuclear fragmentations and dislodged nucleolus showing shifting on one corner of the cell in vacuolated cells.

**Conclusions**: So it might be conclusively inferred that the seeds of *Cascabela thevetia* (L.) Lippold, is highly toxic for germinating plant root cells.

**Implications**: Strict regulation and monitoring is highly needed to keep this ornamental plant from the reach of commercial cash crops including pulse crops for better safety and prevent genotoxicity.

*Key words: Suicide plant, alkaloids, hemolysis, cytotoxicity, chromosomal aberrations, bi- and multi-nucle-ation, micronuclei, nuclear blobs, apoptosis, hemolysis and cellular death*

*Abbreviations:CTSAE: Cascabela thevetia seed aqueous extract, ROS: reactive oxygen species*

From times aeon plants have been in contact with human beings, as direct and indirect associations, imparting their influential impact in development of modern society. Plants are assigned to be the warehouse of different as well as alike phytochemicals which are now being considered as “panacea” for several human ailments in addition to investigational tools/agents that can be exploited for elucidation of complex biochemical reactions/pathways responsible for manifestations of pathological symptoms. There has been an increasing thirst for isolation and identification of varied phytochemicals that can serve as greater leads as “novel drugs’ for customized choice for natural therapeutics for the development of modern medicine. Only a small proportion of medicinal plants have been subjected to judicious exploration out of this huge diversity of unexplored floral population around the world and that too the proper identification and vis-a-vis bioactivity-guided purification and elucidation of their mechanism of action on target organisms has had not attained a commendable statehood yet, as a part of their routine biological and pharmacological screening journey. Moreover, random screening using different model organisms viz, plants, animals, microbes etc., *in vitro* ought to be employed to carry out their diverse mode of actions for a class of similar group of compounds, as crude/bulk drugs, which might open up new vistas of optimistic templates for discovering potent drugs of substantive activity. Now a day’s exploitation of not-so-popular/poisonous plants for this purpose has been in upsurge around the globe for up-gradation of present knowledge base in addition to increase the already popular list of phytochemicals replacing with some newer candidates as potent target drugs to expedite the never ending search for befitting drugs of therapeutic potentialities (Lambert *et al*, 1996).

*Cascabela thevetia* (L.) Lippold, a well-known plant of family Apocynaceae, popular as Yellow Oleander, is cultivated as an ornamental tree and also as a road divider in India (Deb 1993). The plant does not need any form of care or maintenance and thrive nicely in extreme conditions of heat and cold climates (Rajhans *et al.*, 2019) demanding minimum water during the growing stage. It blooms thrice a year yielding numerous fruits with 2-4 flat gray seeds which can be the source of half a litre of oil from nearly 1 kg of dry kernel (Mondal *et al.*, 2016). The seeds are the most poisonous part of this plant than other parts of plants. This plant attains upto 10-18 feet hight having the linear leaves, 13 to 15 cm in length, which remain spirally arranged in the stem (Moni *et al.*, 2014). The various parts of the plant is reach in active constituents of which the leaf has 0.07%, Fruit 0.045%, Seeds 4.8% and the milky sap contains 0.036% of the cardiac glycosides (Thevetin) respectively being responsible for the major causes of intoxication to almost all the vertebrate animals (Kohls *et al.*, 2012). Yellow Oleander is a rich source of phytoconstituents (Rajbhar and Kumar 2014) viz., Alkaloids, glycosides, saponins, flavonoids, and phenolic compounds in addition to fixed oils and fats, and tannins (Kumar *et al.*, 2011). A wide array of pharmacological activities have been reported from time to time which encompassed anti-HIV, anti-inflammatory, antidiarrhoeal, antimicrobial, and cytotoxic activities in addition to anti-spermatogenic, anti-termite, antifungal, anti-oxidant, antimicrobial activities and being in use to treat various human ailments, including diabetes, liver toxicity, fungal infection, microbial infection, inflammation, pyrexia and analgesic activities (Sharma *et al.*, 2022). The latex of *T. peruviana* is rich in metabolites as a source of cytotoxic drugs for fighting cancer ( Al-Rajhi *et al.*, 2022; El-Sawi *et al.*, 2020).

In addition to substantive reports on Cascabella poisoning on human beings there has been a recent report on attenuating effects of *Cascabela thevetia* fruits (MECT) in methanol extract on developmental toxicity and behavioral safety in zebrafish embryos and Haldar *et al*. (2015), could elucidate that *Cascabela thevetia* in zebrafish embryo found to be non toxic at lower doses in laboratory conditions. However, at a higher dose, Cascabella is highly toxic to developing embryos and that had been showing dose dependency. However there has been a contrastingly different but interesting findings (Phuse and Khan, 2018) which reported that flower extracts are less toxic to the human erythrocytes and inhibits erythrolysis owing to its strong flavonoid content. So it can be clearly deciphered that different parts of same plant contains contrastingly different phytoconstituents

which could lead contrastingly opposite pharmacological actions in both *in vitro* and *in vivo* models.

Preclinical assessment for the purpose of safety for human consumption is absolutely necessary for the general principles of Pharmaceutical toxicology especially in this era of synthesized polyherbal formulations. From the point of commercial use of polyherbal extracts (containing complex mixture of biologically active compounds) as potential source of future drugs, an assessment of cytotoxicity/mutagenicity, especially determining the genotoxic end points becomes absolute necessity to pinpoint their narrow dose for safe consumption (Celik 2012). potential toxicity and popularity of different plants in traditional system of medicine is to be determined first and *Cascabela thevetia* is having so many different traditional uses, evaluation of its toxicological parameters (especially from agricultural point of view) becomes also judiciously imperative to gain higher insight into toxic casualties using higher model plant systems. Based on literature review it was found that investigations on developmental toxicity and lethality bioassays are really few (Siby *et al.*, 2020) on dried fruits of *Cascabela thevetia* affecting higher plant development (from seed germination to chromosomal/genome toxicity). Single report is available till date reporting effects of *Cascabella* seed on genetoxicity of commercial pulse crops except *V. faba* (Basu and Tripura, 2021). In both the cases it was reported that *Cascabela thevetia imparted* remarkable cytotoxic activity in terms of gentoxicity in *Allium cepa* and *Vicia faba* L.

Higher plants, like *Allium cepa* L. and *Vicia faba* L., are recommended to be used for bioassay-guided genotoxicity evaluation of plant extracts (Leme and Marin-Morales 2009; Kanaya *et al.,* 1994). These plants are thought to be the most dependable in vivo test systems, and their use would corroborate positive correlations (Bonciu *et al.,* 2018) with other well-known model systems (Grant and Salamone 1994) in a timely, convenient, and affordable manner. However, the chromosomal features *Lathyrus sativus* L., (relatively large chromosomes, less in number with stable karyotype, 2n=14) would allow (Adhikari 2019; Adhikari *et al.*, 2020) easy and repeatable identification of abnormal mitotic phases and chromosomal aberrations after genotoxic sensitization. Cytogenotoxic end points would result in increase and decrease in MI compared to control (Leme and Marin-Morales 2009) which could be visualized by numerical scoring and visual microscopic accounting of structural and numerical changes in chromosome identified as CA (Leme and Marin-Morales 2009; Fiskesjo€ 1985; Bonciu et al. 2018) in addition to ready frequency of MN as an easy cellular marker to assess ecotoxic actions of different classes of xenobiotics (Bonciu *et al.,* 2018; Younis *et al.,* 2019).

Therefore, the present study was undertaken to assess the extent of genotoxic potential and elucidation of the mode and mechanism of action of toxic phytotchemcials present in aqueous extract of *Cascabella thevetia* dried seed in *Allium sativum* L.*,* and *lathyrus sativus* L., root tip cells (*in vivo*).

## MATERIALS AND METHODS

**Experimental materials:**

**Collection of the samples and experimental design for test material treatments:**

Certified seeds of *Lathyrus sativus* L. (variety Mahatora), was procured from the seed testing officer, State Seed testing Laboratory, Govt of West Bengal, India.

Fresh and young seeds of *Cascabela thevetia (L.)* were collected from adjoining areas of Hooghly Mohsin College, Chinsurah, Hooghly, washed thoroughly under tap water, patted on filter paper to semidryness and then kept under bright sunlight during the summer months of April and May 2022.The dried seeds were grineded to find powder and kept in air-tight clean glass jars for future use. Required amount of powdered sample was brought to boiling for 10 min with adequate amount of distilled water. Infusion of the powdered plant material (dried fruit powder) in different concentrations (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively) as these concentrations could produce visible alterations in both physiological (germination) and cytological changes (visible chromosomal aberrations) in different plant models that can be scored for visualization and interpretation of accomplished results to be considered for toxicity studies (Patel *et al.*, 2019).

The decoction was filtered in Whatman 1 filter paper for experimental use as *Cascabela thevetia* (L.) Lippold seed aqueous extract (CTSAE). Distilled water was used as negative control.

**Alkaloid extraction:**

To detect the potential alkaloids, extracts were prepared from air-dried and powdered form of *Cascabella* seeds to be used in GC/MS analysis. Plant material (500 mg) was separately extracted 3 times with methanol (5 mL) at room temperature. The solvent was evaporated under reduced pressure, the residues were dissolved in 10 mL of 2% sulfuric acid, and the neutral compounds were removed with diethyl ether (3×10 mL). The acidic aqueous phases were basified with 25% ammonia to pH 9-10 and the alkaloids were extracted with chloroform (3×10 mL). The combined chloroform extracts were then dried over anhydrous sodium sulfate, filtered, and the organic solvent was distilled *in vacuo* to afford the alkaloidal extract. The obtained extracts were used for GC-MS analysis.

**GC-MS analysis:**

The GC-MS analysis was performed using GLC-MS; Shimadzu QP 5050 A (Shimadzu, Kyoto, Japan) operating in electron impact mode (EI, 70 eV). The oven temperature was programmed as 80°C for 1 min, 80-250°C (10°C min-1), 250°C for 2 min, 250-300°C (10°C min-1), and a 10 min hold at 300°C. The injector temperature was 250°C. Helium was used as carrier gas at a flow rate of 0.8 mL min-1. A TR-5 MS column (30 m×0.25 mm×0.25 µm) was used. The extracts were dissolved in methanol (1 mg of extract in 500 µL of methanol). All injections were run in splitless mode. The spectra of co-eluting chromatographic peaks were investigated and deconvoluted by the use of Xcalibur (version 2.07; Thermo Fisher Scientific San Jose, CA, USA). The compounds were identified by comparing their mass spectral fragmentation with standard reference spectra from the NIST 05 database (NIST Mass Spectral Database, PC-Version 5.0 (2005), National Institute of Standardization and Technology, Gaithersburg, MD, USA), or applying co-chromatography with previously isolated authentic standards and in comparison with data obtained from the literature. The percentage of total ion concentration for each compound is given in Table 1. The area of the GC-MS peaks depends both on the concentration of the corresponding compound and on the intensity of their mass spectral fragmentation. Moreover, they can be used for a relative comparison of alkaloids.

**Evaluation of genotoxicity in meristematic cells of *A. sativum* L. and *Lathyrus sativus* L.,**

The seeds of *Lathyrus sativus* L., were surface disinfected with 1% sodium hypochlorite for 5 min and vigorously rinsed with distilled water. Then they were incubated (24hours) with different concentrations of corresponding to (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively) (CTSAE) for 24h (imbibitions/seed priming). Control was set up by seed immersion in distilled water. Petri dishes were then arranged randomly in a BOD incubator at 22 ± 2 °C with a 12:12 h light: dark photoperiod (Souza *et al.*, 2017) where the treated seeds were placed on moist filter paper in covered Petri dishes in order to germinate. (Adhikari *et al.*, 2020). Mitotic index and chromosomal aberrations in metaphase and anaphase plates were studied using a light microscope under oil immersion. From each slide, minimum of 100 cells were scored and mitotic index was calculated. Chromosomal aberrations such as chromosome fragments (F), precocious separation (SP), stickiness (STC), bridge formation (Br), c-mitosis (C-m), micronuclei, etc were studied in a minimum of 100 cells per slide and expressed in percentage (Adhikari, 2019). All stages were examined at 40 x and under oil immersion objective using a oil immersion lens, 100/1.25 eyepiece of a compound microscope (Olympus CH20i microscope) fitted with CMOS Camera (IS 500, 5.0 MP) and its attachment with a computer with the aid of VIEW 7 image analysis software.

Fresh healthy garlic (multiclove*; Allium sativum* L.,) of 20±15 g was chosen and procured from the local market. The cloves of the *Allium sativum* L., were gently separated by hand tearing and the old roots and scales were carefully removed. The denuded cloves were placed over sand pots moistened with water sprinkling were placed in an isolated space at 25 ± 1ºC for the root induction. The freshly emerged roots of about 0.5-1 cm length were treated by placing the rooted cloves on test tubes containing concentrations of 5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml (CTSAE) respectively for 24h. Distilled

water was used as a negative control. Cytological investigations were carried out following the protocol of Adhikari (2019).

**1. Genotoxicity in meristematic cells:** This was evaluated according to de Souza et al. (2022), Approximately, 2,500 meristematic cells were analyzed per treatment. Cytotoxicity was evaluated based on the mitotic index (MI) according to the formula:

$$MI = \frac{\text{Number of cells in division}}{\text{Total number of cells observed}} \times 100$$

Genotoxicity was analyzed based on the number of cells carrying chromosomal and nuclear aberrations, such as nuclear buds, anaphasic and telophasic bridges, and chromosomal losses and breaks. The genotoxicity index (GenI) was calculated using the formula:

$$\text{Gen I} = \frac{\text{(No of cells showing chromosomal abnormality + Nuclear buds+ nuclear breakage)}}{\text{(Total no of cells counted)}} \times 100$$

$$\text{Mitotic Inhibition} = \frac{\text{(Mitotic index in Control- Abnormal Mitotic index after treatment)}}{\text{(Mitotic index in Control).}} \times 100$$

**2. The frequency of micronuclei (FMN)** was determined by assessing the number of cells containing MN:

FMN= (No of cells showing MN) ÷ (Total no of cells counted) x 100.

The presence of MN can be used as a biomarker for genotoxicity and chromosomal instability-related events. When this damage is not repaired, it has the capacity to produce mutagenic effects.

**3. Detection of Morphological Characters for cell death, computation the Percentage of Dying Cells:** We chose nucleus migration from centre to margin of cell wall, condensation, vacuolation of cytoplasm, nuclear fragmentations as characteristic hallmarks of dying cells. Nucleus margination is displacement of nucleus in a cell wall margin (Behboodi and Samadi, 2002)

Percentage of Dying Cells= (No of cells dying or dead cells) ÷ (Total no of cells counted) x 100.

**4. Observation and manual scoring of cell dimensions of Giant cells with vacuolated cytoplasm:** to detect the extent of protoplasmic shrinkage in germinating root cells roots were transferred in a incubation solution (containing 0.1 M sodium phosphate buffer pH 7.2 containing 0.3 M sucrose with 6µM neutral red) for 30 minutes (Curtis and Wolpert, 2004). The cells roots were then hydrolyzed in 1N HCl and counterstained with 2% acetoorcein and safranine solution. The root tips were then squashed in 45% acetic acid and Photographs were taken from all the morphogenic regions of the root tip (in x 100 magnification) and cell dimensions like shrinkage areas, legth, breadth and cytoplasmic areas were observed and scored (Behboodi and Samadi, 2002).

All the histograms were prepared using MS-EXCEL.

## RESULTS

**GC-MS analysis:**

A number of alkaloids was found present in *Cascabella pepper* seeds powder extracts (CTSAE ) which have been identified and quantified by GC-MS technique, indicating that this method for chemical analysis is useful and reliable for studies on the alkaloid metabolism in this family. The alkaloid patterns of the samples were normalized and presented as a % of individual compounds in the total alkaloidal mixture (Table 1). Totally, 11 compounds with mass spectral characteristics of Amaryllidaceae alkaloids were detected in the extracts of the *Cascabella* seeds. The identified compounds possessed various Amaryllidaceae alkaloid skeleton types including lycorine, galanthamine, and crinine types and additionally indole alkaloid and another base hordenine. Hordenine is not typical for plants of the family Amaryllidaceae.

**Detection of abnormal mitotic index in *Allium sativum* L., and *Lathyrus sativus* L., root tip cells:**

The mitotic index (MI) values of both normal (dividing cell showing Prophase, Metaphase, Anaphase, and telophase;, since prophase and prometaphase are difficult to distinguish, all cells were classified as prophase) and

abnormal MI in treatment groups (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively) with the total number of cells observed with normal mitotic division and nondividing (out of treatement regime) in the total number of cells viewed per slide of extract concentrations was quantified and tabulated (Table 3) for the evaluation of the mitotic index and mitotic depression. A quantitative evaluation was also done for the number and type of chromosomal aberrations observed (Chromosome aberrations were observed and scored in the following categories: anaphase-bridges, chromosome or chromatid fragments and laggards in dividing cells, mitotic depression, C-mitosis/metaphase (no spindle fibers), vagrant chromosomes, micronucleus, binuclei, tri-nuclei and tetra-nuclei in the interphase stage, and sticky chromosomes; the types of chromosomal abnormalities were evaluated and taken into account for the evaluation in the cytogenic study, as a cytogenotoxic bio-marker) in the total number of cells viewed per slide of extract concentrations (Tables 3) for both *Allium sativum* L., and *Lathyrus sativus* L. In *Lathyrus sativus* after 96 h of germination there were gradual blackening and necrosis like symptoms occurred (population bio-marker of tissue death) could be visualized which might be the poisoning effect of the extract. A large number of elongated ghost cells and giant cells with strap-like nucleus were counted in the medium to higher range of (20mg/ml, 30 mg/ml and 40 mg/ml pretreatments) which were the direct outcome of alteration in the cellular metabolic process with time lapsed. In the lower concentrations (5 mg/ ml and 10 mg/ml) treated *Lathyrus sativus* treated germinating root tips there was an array of chromosomal aberrations in both dividing cells and nuclear aberrations in interphase cells. The total number of bi-nucleate, tri-nucleate and tetra-nucleate cells and multi-vacuolated resting nuclei were observed that were proportionately high in comparison to micronuclei, nuclear blobs, nuclear bridge formation which indicated the mitostatic/ spindle fibre disrupting action (stathmokinesis) of the toxic alkaloids present in the aqueous extract of CTSAE. In the higher concentrations (20 mg/ml and 30 mg/ ml) treatment there establishment of highest frequency of chromosomal aberrations, like stickiness and metaphasic clumping, metaphasic ring with early separation, early and late chromosomal speataions, chromosomal arm breakage forming acentric fragments, polyploidy cells with C-mitosis, giant ghost-like cells had been tabulated (Table 3). At the utmost highest concentration (where the visible symptoms of chromosomes could be encountered) i.e. 40mg/ ml pretreatment, maximum cells were showing apoptotic symptoms where complete erosion of chromosomes showing karyorrhexis (in resting cells) and karyolysis (dissolution of nuclear materials) in both dividing and giant cells having big central vacuoles. The nucleus was lobbed, shifting towards the corners and fragmented showing early apoptotic/necrosis like pathological manifestations.

Table. 1. List of isolated alkaloids present in the CSTAE after GC-MS.

| Compound | R.T. in min | % Total ion concentration | m/z peaks |
|---|---|---|---|
| Hordenine | 11.2 | 4.21 | 121,107,91,77,58 |
| Ismine | 20.13 | 0.08 | 238,211,196,168 |
| Trisphaeridine | 21.05 | 1.22 | 222,167,138,111,69 |
| Crinine | 21.4 | 0.05 | 270,254,228,214,199,185,150,122 |
| Galanthamine | 21.6 | 0.55 | 286,270,244,230,216 |
| Anhydrolycorine | 22.98 | 1.12 | 250,224,192,191,96 |
| Assoanine | 23.56 | 0.54 | 266,250,222,207,193,180 |
| Lycorine | 25.28 | 0.99 | 268,250,228,227,226,147 |
| Galwesine | 26.78 | 0.31 | 207,155,140,112,96,73 |
| Galanthine | 24.54 | 43.2 | 316, 284, 268, 266, 244, 243, 242 |
| Incartine | 25.1 | 22.6 | 332, 259, 258, 244 |

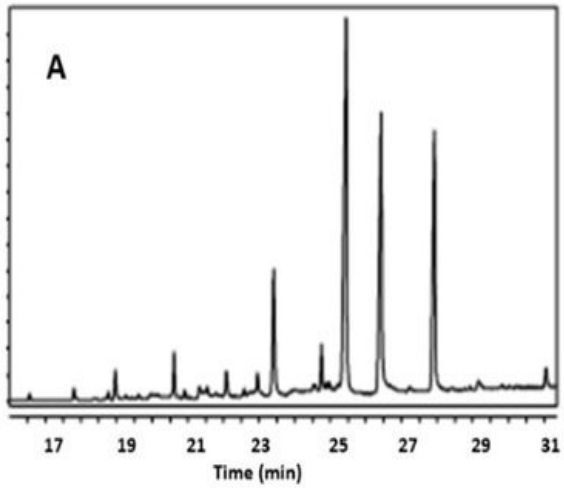
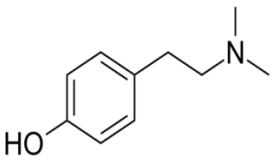
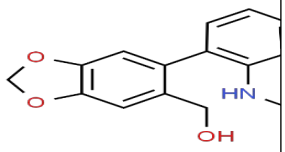
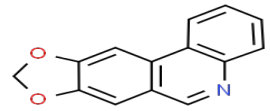
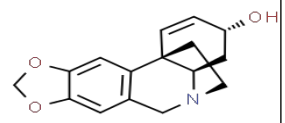
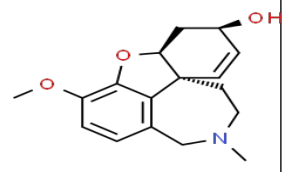
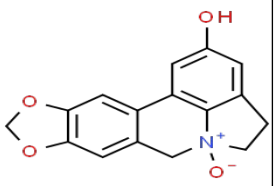
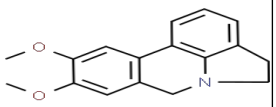
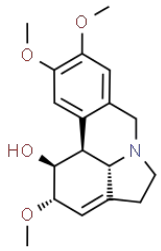
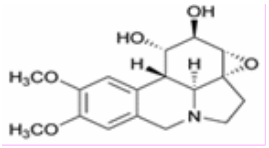
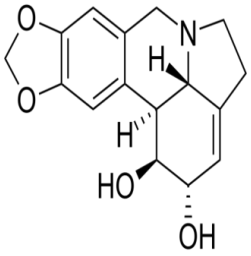
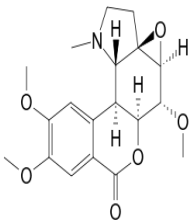



**Figure 1.** GC-MS chromatogram of the alkaloids Cascabela thevetia (L.) Lippold seed aqueous extract (CTSAE)

**Table 2:** Chemical characterization of the isolated alkaloids in CTSAE after GC-MS

| Name | Mol.formula and weight | Chemical characteristics and origin | Chemical structure |
|---|---|---|---|
| Hordenine | $C_{10}H_{15}NO$, IUPAC Name:4-[2-methylamino)ethyl]phenol, 165 | Hordenine is an amphoteric molecule containing both basic (amine) and acidic (phenol) functional grou. It is mildly soluble in water and highly soluble in ethanol, ether and chloroform. |  |
| Ismine | $C_{15}H_{15}NO_3$ IUPAC Name: 6-[2-(Methylamino)phenyl]-1,3-benzodioxole-5-methanol , 257 | Ismine is a member of biphenyls and a natural product found in *Lapiedra martinezii* and *Lycoris squamigera*. It is soluble in water and organic solvents. |  |
| Trisphaeridine | $C_{14}H_9NO_2$ IUPAC Name: 8,9-(Methylenedioxy) phenanthridine, 223 | Trisphaeridine is a member of phenanthridines andnatural product found in *Pancratium trianthum* and *Crinum yemense*. It is insoluble in water and organic solvents. |  |
| Crinine | $C_{16}H_{17}NO_3$ IUPAC Name: (3α)-1,2-Didehydrocrinan-3-ol, 271 | Crinine is a natural product belonging to the class of alkaloid found in *Calostemma purpureum* and *Crinum americanum*. It is water soluble. |  |
| Galanthamine | $C_{17}H_{21}NO_3$,IUPAC Name: (4aS,6R,8aS)-4a,5,9,10,11,12-Hexahydro-3-methoxy-11-methyl-6H-benzofurol [3a,3,2,-ef] [2]benzazepin-6-ol, 287 | Galantamine is a tertiary alkaloid which can reversibly, inhibit competitively the acetylcholinesterase (AChE) enzyme, sparingly soluble in water. |  |

| | | | |
|---|---|---|---|
| Anhydrolycorine | $C_{16}H_{13}NO_2$, IUPAC Name: 5,7-Dihydro-4H-[1,3]dioxolo[4,5-j] pyrrolo[3,2,1-de] phenanthridine, 251 | Anhydrolycorine is a natural product found in *Leucojum aestivum, Narcissus papyraceus,* and *Narcissus tazetta* and is sparingly soluble in water. |  |
| Assoanine | $C_{17}H_{17}NO_2$,IUPAC Name: 4,5-Ethylene-8,9-dimethoxy-4,5-dihydro-7H-phenanthridine, 267 | Assoanine is a member of phenanthridines class of natural product found in *Narcissus jacetanus* and *Narcissus assoanus.* |  |
| Galanthine | $C_{18}H_{23}NO_4$, IUPAC Name:(1α,2β)-2,9,10-Trimethoxy-3,12-didehydrogalanthan-1-ol, 317 | Galanthine is a natural product found in *Lycoris sanguine* and *Hippeastrum puniceum.* |  |
| Incartine | $C_{18}H_{23}NO_5$ IUPAC Name:Galanthan-1-ol, 3,12-epoxy-2,9,10-trimethoxy-, (1.alpha., 2.beta.,3.alpha.)- 333 | Incaritin is a natural product found in *Epimedium diphyllum*, *Epimedium wushanense*, |  |
| Lycorine | $C_{16}H_{17}NO_4$, IUPAC Name (1S,2S,3a$^1$S,12bS)-2,3a$^1$,4,5,7,12b-Hexahydro-1H,10H-[1,3]dioxolo[4,5-j]pyrrolo[3,2,1-de]phenanthridine-1,2-diol, 287 | Lycorine is an alkaloid found in cultivated bush lily (*Clivia miniata*), surprise lilies (*Lycoris*), and daffodils (*Narcissus*) and Amaryllidaceae species showing highly poisonous actions after ingestion with certain quantities. |  |
| Galwesine | $C_{19}H_{23}NO_6$,IUPAC Name: (3aR,4aS,5R,5aS,11bS,11cS)-5,9,10-trimethoxy-1-methyl-1,2,3,4a,5,5a,11b,11c-octahydro-7H-isochromeno[3,4-g]oxireno[2,3-d]indol-7-one, 361 | Galwesine belongs to the family of Amaryllidaceae also commonly known as “snowdrop”. |  |

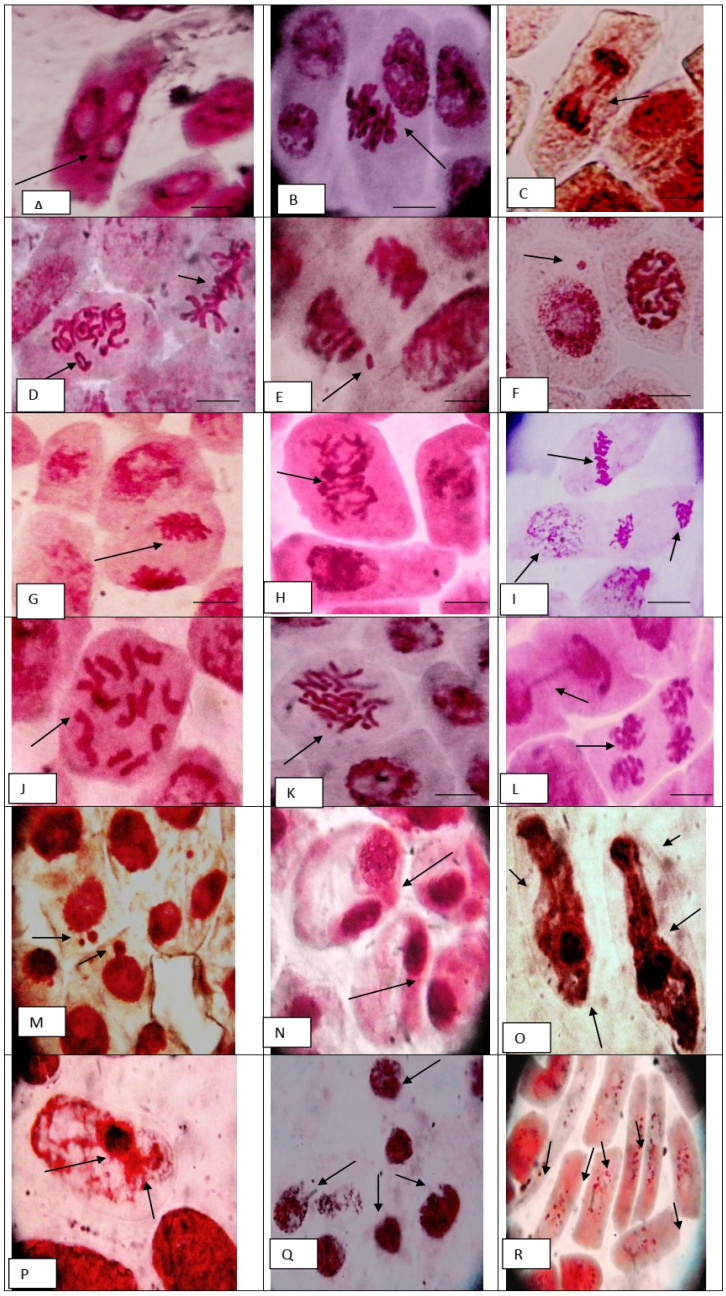
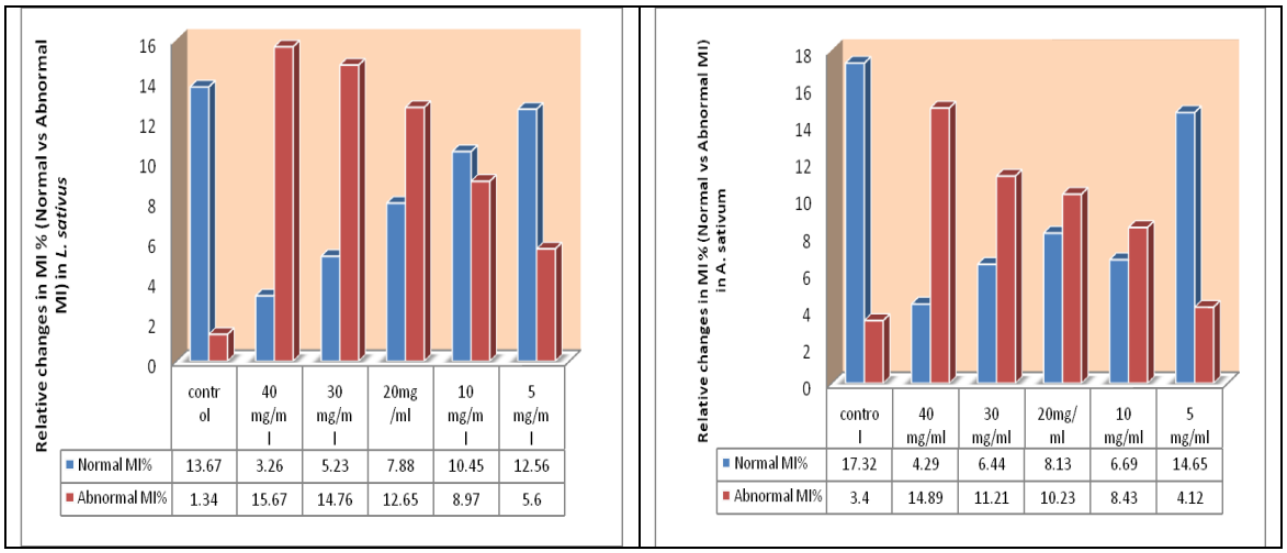
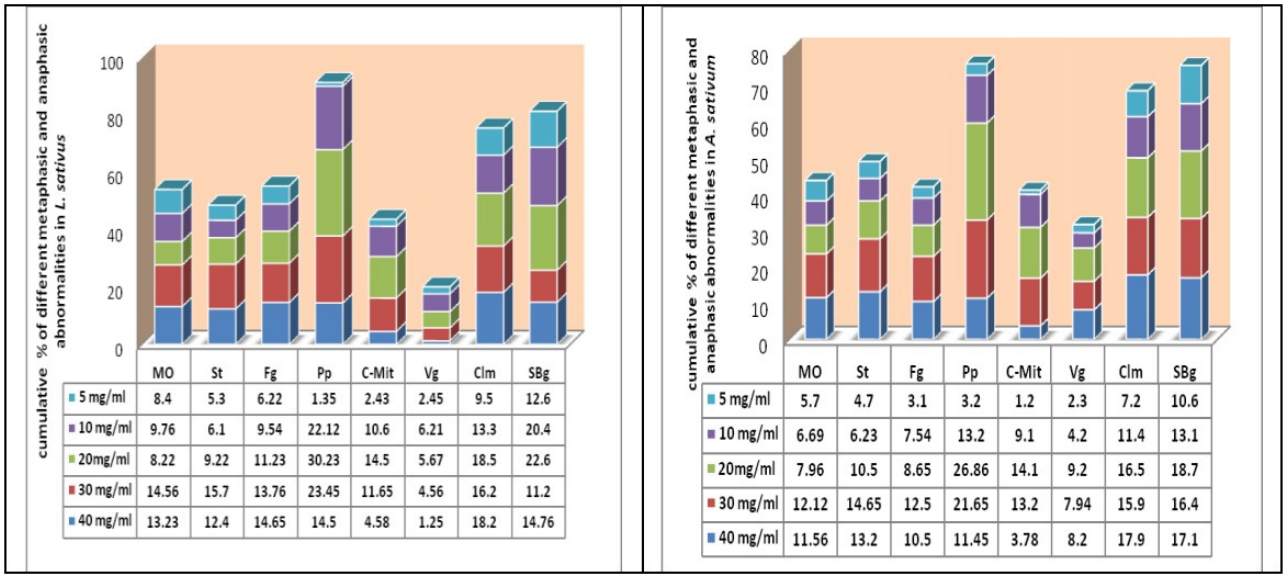



**Figure 2.** (A-R): showing the cytogenotoxic effects of CTSAE pretreatments (24h) *Lathyrus sativus* L. germinating root tip cells with increasing concentrations (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively). A= Binucleate cells with vacuolation (5mg/ml), B= Somatic diad with prophase and metaphase after 5 mg/ml pretreatment, C= Anaphasic Sticky Bridge after 5mg/ml pretreatment, D= Early ball metaphase with ring formation, pole to pole metaphase after 10 mg/ml pretreatment, E= Disturbed anaphase with arm breakage after 10 mg/ml pretreatment, F= Micronuclei at interphase and pulverized chromatin after 20 mg/ml pretreatment, G= micro and macrcell formation with stellate anaphase after 20 mg/ml pretreatment, H= equatorial anaphasic separation after 20 mg/ml pretreatment, I= Giant cells with metaphasic hypercondensation; chromosomal erosions and stellate anaphase after 20 mg/ml pretreatment, J= c-Mitosis after 20 mg/ml pretreatment, K= Polyploidy after 20 mg/ml pretreatment, L= Interphasic nuclear bridge formation and “Somatic Tetrad” formation after 30 mg/ml pretreatment, M= nuclear budding at interphase nucleus after 30 mg/ml pretreatment, N= Giant cells with early symptoms of necrosis, nuclei migrated to corners with cytoplasmic disappearance after 40 mg/ml pretreatment, O= Giant cells with cytoplasmic shrinkage away from cell walls leading to apoptosis after 40 mg/ml pretreatment. P= Apoptotic cells completely disintegrated protoplasm with disappearing nucleus after 40 mg/ml pretreatment. Q= Interphase nuclei showing chromatinal erosion, karyorrhexis at onset of apoptosis after 40 mg/ml pretreatment. R= Complete karyolysis in all the cells after 40 mg/ml pretreatment.



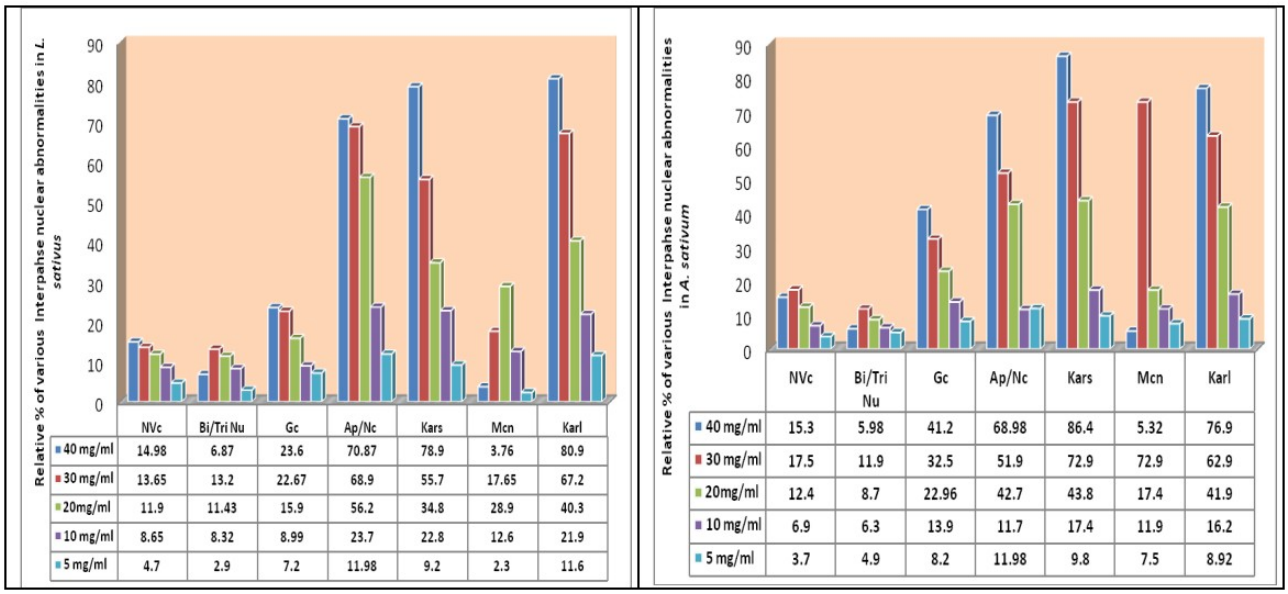

**Graph 1:** Histogram showing relative change in MI % (Normal vs Abnormal MI) after pre-incubation (24 hrs) with CTSAE in *L. sativus* L. and *A. sativum* L., root tip cells.

According to the graphs, pre-exposure to CTSAE at dosages of 40, 30, 20, and 10 mg/ml resulted in significant changes in MI%, i.e., attenuation of abnormal MI and reduction of the normal rate of MI in both plant systems. The Graph I (comparative histograms) demonstrated that, in contrast to *A. sativum L.* germinating root cells, *L. sativus* root tips displayed a higher aberrant MI% in the doses described above. While *A. sativum* could withstand the toxic allelochemicals (alkaloids) in CTSAE at lower doses, resulting in less abnormal MI% (11.21 and 10.23 %, respectively) than *L. sativus* L., root tip cells (14.76 and 12.65%), the abnormal MI% were nearly the same at the highest dose (40 mg/ml) (15.67 and 14.89%, respectively). This essentially depicts the sensitivity of *L. sativus* L. cells to the toxic alkaloids found in CTSAE, which readily penetrated the seed coat of the plant and caused toxicity in the root cells that were just beginning to germinate.




**Graph 2:** Histogram showing cumulative % of different types of metaphasic and anphasic chromosomal aberrations after pre-incubation with CTSAE in *L. sativus* L. and *A. sativum* L., root tip cells. Mo= misorientation, St=stickiness, Fg=fragmentation, Pp=polypolid like cells, C-Mit=C-mitosis, Vg= acentric vagarant and ring chromosome, Clm=clumpting, SBg=sticky bridge.

Comparative histograms (Graph:2) revealed that, in contrast to *A. sativum* root cells, there is a greater propensity for toxicity in germinating *L. sativus* L. root tip cells due to the direct action of CTSAE alkaloids, even after 72 hours of germination and formation of varied percentages of different metaphasic and anaphasic chromosomal aberrations. However, an intriguing discovery emerged from this investigation: in both *L. sativus* L. and *A. sativum* L., root tip cells showed a significant percentage of polyploid-like cells and C-mitosis, especially at a dose of 20 mg/ml. This observation clearly indicates that the multivariate levels of toxic alkaloids present in CTSAE could disrupt spindle fibres. Remarkably, the relative percentages of polyploid-like cell populations formed in *L. sativus* L. root cells and *A. sativum* L. root tip cells were significantly larger (30.23 % and 26.86%, respectively) in the former case. In contrast, the C-mitosis percentages were essentially same. *L. sativus* L. and *A. sativum* L. root tip cells' varying susceptibilities to the spindle-fibre disturbing effects of CTSAE alkaloids may be explained by this relative shift in polyploidy, which involves differential sensitivity of both the two plant cells towards phytotoxicity.




**Graph 3:** Histogram showing Relative % of various types of interphase nuclear aberrations after pre-incubation with CTSAE in *L. sativus* L. and *A. sativum* L., root tip cells. Nvc=vacuolated nucleus, Bi/Tri Nu= Bi and Tri-nucleation, Gc= Ghost Cell with strap nucleus, Ap/NC=Apoptotic/Necrotic cells, Kars= Karryorrhexis, Mcn=Micronuclei, Karl=karyolysis

The direct genotoxic efficacy of CTSAE at 40, 30, 20, and 10 gm/ml preexposure resulted in varying percentages of interphase nuclear abnormalities in both plant root cells, according to comparative histograms (Graph: 3). Ghost cell development with strap nucleus production was observed at higher levels in *A. sativum* L. cells (41.2%) compared to *L. sativus* L. cells (23.6%). Conversely, the populations of apoptotic and necrotic cells were higher in the root tips of *L. sativus* L. However, the proportion of cells exhibiting Karryorrhexis (86.4%, respectively) in *A. sativum* L. was significantly higher than that of *L. sativus* L. root tip cells. This also provided a compelling explanation for the two plant systems' differing sensitivity to the same class of toxic alkaloids, which could be related to the two different plant root cells' varying levels of antioxidant status, osmolyte balance and physiological vigour, and genomic stability against allelochemicals.

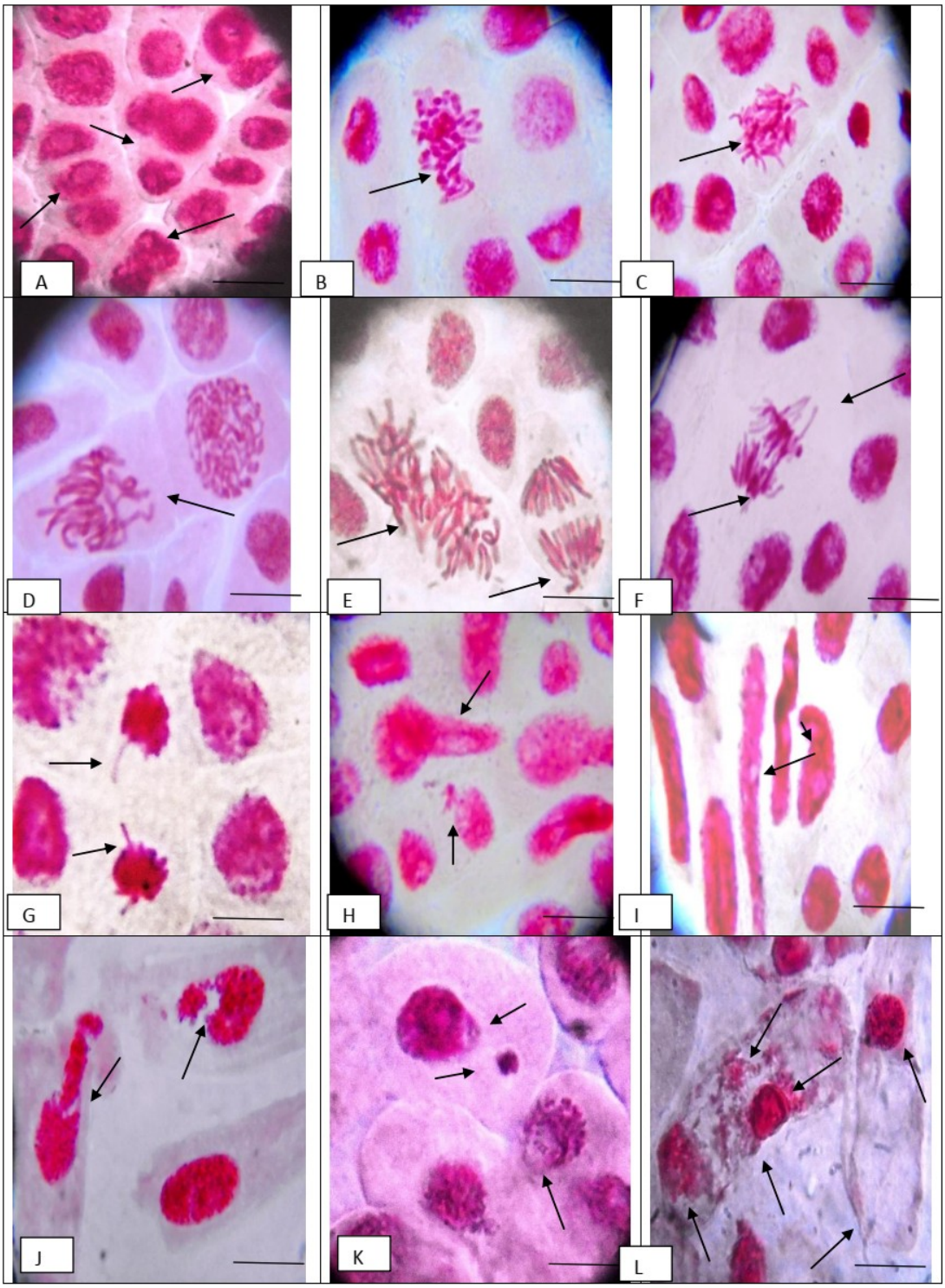



**Figure 3.** (Plates A-L): showing the cytogenotoxic effects of CTSAE pretreatments (24h) in *Allium sativum* root tip cells with increasing concentrations (5mg, 10 mg, 20 mg, 30 mg and 40 mg/ ml respectively), A= Bi and tri=nucleate cells with vacuolation (5mg/ml), B= Megadikaryon with early ball metaphase with multiple rings after 5 mg/ml pretreatment, C= Coagulated early sticky anaphase after 10mg/ml pretreatment, D= late prophase chromatinal gap after 10 mg/ml pretreatment, E= polyploidy cell after 20 mg/ml pretreatment, F= Stathmoanaphase with early movement after 20 mg/ml pretreatment, G= stellate anaphase with late movement after 30 mg/ml pretreatment, H= Nuclear budding and nuclear tails in strap cells after 30 mg/ml pretreatment, I= Giant cells with strap nuclear lesions after 40 mg/ml pretreatment, J= karyorrhexis after 40 mg/ml pretreatment, K= Macro and Micronuclei formation, nuclear erosion after 40 mg/ml pretreatment, L= Apoptotic cells completely disintegrated protoplasm with disappearing nucleus and shrinking protoplasm away from cell wall after 40 mg/ml pretreatment,

## DISCUSSION

Higher plant bioassays to detect the synthetic and natural samples present in the natural environment employing *Allium cepa, Vicia faba*, have been intensely and immensely employed across globe because of the simplicity, cost-effectiveness, ruggedness and acquired reproducibility of results time to time (Firbas and Amon 2014). In addition, lesser attentions have been garnished towards employing *Allium sativum* L. (U¨nyayar *et al.*, 2006) and *Lathyrus sativus* L., as equipotent plant model systems to evaluate gentoxicity that had been garnering promising results with accurate manifestations of toxicological responses after treatment with a varied range of environmental pollutants e.g., pesticides (Onuminya and Eze, 2019), industrial effluents (Soumyashree *et al.*, 2014), nanoparticles (Dogra *et al.*, 2020), heavy metals (Adhikari, 2021, Ghosh *et al.*, 2020) including phytotoxic plant extracts (Guadalupe Velázquez-Vázquez *et al.*, 2022). Moreover, higher plants possess some advantages over other organisms in certain modalities owing to their possession of large chromosomes, low chromosome numbers, stable ploidy where the somatic root meristematic cells multiply rapidly giving high proportion of metaphasic cells (Leme and Marin-Morales, 2009). *Allium cepa*, although available in the market throughout the year, has had the tendency/inability for rooting during prevailing summer months with scanty rooting where the whole onion bulb needs to be sacrificed for study of single mutagen thereby often thus makes hindering consequences for data collection which demands to look out for an alternately effective plant for bioassay studies. As a popular alternative, *Allium sativum* L., the common garlic belonging to the family Alliaceae and genus *Allium*; contains larger chromosome compliments with same number as *Allium cepa* thus have been in use as an alternative, popular and sensitive test system for the evaluation of environmental pollutants and screening of chemicals with genotoxic effects, (Saxena *et al.*, 2009; Hemavathi *et al.*, 2015). *Allium sativum* can be split into sinle clove, unlike *A. Cepa*; thus making this resource nicely adjusted to different laboratory set ups for assay models as the clove's roots have been subjected to singular treatments nicely as against the whole bulbs of *Allium cepa* L. On the other side, Grass pea (*Lathyrus sativus* L.) is a Neolithic plant is a robust legume crop (Lambein *et al.*, 2019) that is climate resilient pulse with easy availability, low-priced, easy-to-store grains with very good germination potential throughout the year having stable bimodal karyotype 2n=14, has been found to be an excellent model for plant based genotoxicity assays. Different varieties are available throughout the year which upon treatment with different test chemicals augment almost all the biochemical and cytogenotoxic responses, sometimes much superior than other pulse crops (Adhikari 2019) providing the much option choosing than the standard alternatives i.e., *Vicia faba* and and *Allium cepa* L.

In the present study there was commencement of differential and varied levels of cytotoxic and genotoxic manifestations of CTSAE on both i.e., *Allium sativum* and *Lathyrus sativus* L. in terms of clastogenic and aneugenic symptoms along with variable frequencies of abnormal mitotic index has been regarded as the *prima facie* impression to estimate the test compound's CTSAE ability to induce cytotoxicity, gentoxicity and mutagnecity in the model test systems. Allium roots would differentially activate promutagens or gentoxic substances with the help of mixed oxidase enzyme systesm (Adhikari *et al.*, 2014; Abdel Migid and Abdelrahman 2013), which in turn amplifies inhibition of rooting with stunted growth (cytotoxic marker) and root wilting/necrosis (genotoxicity out of cellular apoptosis). In this experimental system none of the concentrations of CTSAE could produce root wilting/necrosis after 24 hrs of exposure and subsequent recovery. Surprisingly there was an incidence of higher root length promulgation at 10 mg/ml and 20 mg/ml in comparison to control (statistically insignificant although; data not accounted) with continued upto 30 mg/ml test treatment showing uniformity with earlier reports (Verma and van Huyste 1971). After cytological squashing there have been the occurrence of ghost/giant cells, which are highly elongated tapering end cells and strap cells with elongated disintegrated nucleus which are reported to be cytotoxic in nature, which in conformity with earlier reports *Cascabela thevetia* (L.) Lippold and synthetic food colorants treatments in *Allium cepa* (Prajitha and Thoppil 2016; Prajitha and Thoppil 2017). Any physical and chemical mutagenic component might cause cytoplasmic

chrinkage has have the ability to produce giant cells and strap cells which fail to undergo normal karyokinesis (Siby *et al*, 2020). Earlier Furadan and Monosodium glutamate treatement in *A.cepa* root meristem cells had been found to produce huge frequencies of strap cells (Prasath et. al. 2013). Thus it would have been correlated and corroborated that CTSAE would have disrupted the usual cell cycle especially in the 'S' phase, thereby the root tip cell division must have been partially arrested due to the toxic effects of consortium of phytotoxic components (like the flavonoids, alkaloids, saponins, phlobatannins, glycosides, terpenoids, phenolos etc. present per say) in CTSAE. This disruptive action of CTSAE thereby could have augmented the reduction in mitotic index in treatments (Graph: 1), followed by mitotic arrest as a direct outcome of hindered synthesis phase and different frequencies of clastogenic abnormalities in addition to cytoplasmic and nuclear shrinkage and vacuolation followed by nuclear and chromosomal erosions, nuclear disintegrations and intrusions resulting nuclear blebbing and budding, pulverized nucleus during karyorrhexis and servral nuclear bridge formations at anaphase and multinucleate cells without cell plate/nuclear membrane formations (stathmokinesis) during telophase in *Lathyrus sativus* L, (Figure 2, Plates A-R). Within these Giant cells, apart from high frequency of abnormal ana-telophasic cells; there occurred several nuclear aberrations that were of varying types, which included binucleate to trinucleate conditions, mitotic tetrads, nuclear lesions containing double to multiple nuclear lesions, nuclear elongation with fragmentations, nuclear budding and micronucleus formation, hyperchromasia, nuclear erosion, pulverized nucleus, unequal condensation in early prophase, pole to pole metaphase arrangement with a fragment, macro and microcell formation showing unsynchronized chromosomal substages (Figure 3, Plates A-L, Graph: 2 & 3). All these abnormalities within the giant cells are possible "cytological biomarker" conforming the possible DNA synthesis blockage and disruption of spindle formation. This high frequency of strap and giant cells might have been centre for "transduction of apoptotic signals" which could ultimately induce the abnormal/aberrant cells to undergo apoptosis. This high frequency of giant cell formation is indicative of cytological screening and evaluation of mode of action of different synthetic and natural compounds which can readily interfere in cell proliferation showing bioassay-guided carcinogenesis. In this regard CTSAE have been showing giant cell and strap nucleus formation in both *Allium sativum* and *Lathyrus sativus* root tip cells guaranteeing its superiority in terms of further evaluation and purification of phytotoxic compounds in future formulations as antiproliferative drugs.

While doing the preliminary phytochemical analysis did detect the presence of phenolic compounds which would explain the high rates of cells possibly undergoing to apoptois as an outcome of direct antiproliferative effect of CTSAE enumerated by the drastic fall of the MI in both *Allium sativum* and *Lathyrus sativus* L., root tip meristematic cell population with increasing does after seed priming. There had been increase in the micronucleus index in *Channa punctatus* (Table: 4, plate: B, Table:). In addition there was the detection of positive qualitative presence of of saponins (after foam test), Flavonoids (After Alkaline reagent test), tannins (Bragmer's test) and terpenoids (After Salkowski test) detected by the phytochemical screeing of the CTSAE separately in the laboratory (data not included). The presence of same compound has been reported from other parts of the Cascabella tree (like dried leaf, flower extracts) recently by (Sibby *et al.*, 2020). Saponins have a wide structural variability and being reported to be the end-products of the secondary metabolic pathways. With strong antitumoral activity in animal cell lines saponins established that in several cases they could inhibit the cell cycle bringing apoptosis of normally dividing cells (Upadhyay and Singh, 2012). Differnt authors time to time have reported that plant extracts such as *P. leiocarpa* and *P. Myriantha* (Lubini *et al.,* 2008), *Campomanesia xanthocarpa* (Pastori *et al.,* 2013), *Vernonanthura polyanthes* (Amado *et al.,* 2020), *Amaranthus spinosus* (Prajitha and Thoppil, 2017)*, Achyrocline satureioides* (Fachinetto *et al.,* 2007), *Luehea divaricata* (Frescura *et al.,* 2012) and *Sambucus canadensis* (Guadalupe Velázquez-Vázquez *et al.*, 2022) caused a reduction in the mitotic index yielding several aneugenic and clastogenic responses in plant chromosomes when exposed to increasing concentrations of different polar and aploar

solvent extracts, which might be due to the strong presence of alkaloids, flavonoids, polyphenolics and tannins, in varying quantities apart from saponins. The results obtained after FTIR analysis in *Sambucus canadensis could detect varying proportions of* flavonoids which in lower doses did not show inhibition of cell division but in higher doses could effectively inhibit or stimulate the cellular cycle vis-a-vis DNA metabolism. Interestingly studies by Tedesco *et al.,* (2017) effectively established that flavonoids pool in different phytoextracts constituted a consortium of variable proportion of rutin, kaempferol and quercetin, might be imparting varying pharmacological actions, including antiproliferative and anticancer action in animal models. Reporting by Lee and Finn, (2007) propounded that substantive quantities of polyphenols in *Sambucus canadensis* could augment inhibition of cellular division in *Allium sativum.* In this connection, the common view has been established as the genotoxic and antiproliferative activity presented by some plant extracts are the result of the interactions of their different chemical components which are directly dependent on concentration as per Bagatini *et al.* (2009) and exposure duration as per Toloza *et al.* (2006). The large consortium of phyto-constituents present in CTSAE might be responsible for pronounced cytotoxicity and antiproliferative activities (stathmo-anaphase) through visible scoring of apoptosis (clastrogentic and aneugenic responses) in both *Allium sativum* and *Lathyrus sativus* L., root tip cells in the present study cannot also be rule out. Flavonoids have been implicated to induce DNA mutations and chromosomal aberrations other metabolites such as saponins, alkaloids, steroids might be overwhelmingly responsible for the root growth inhibition observed. This can be further corroborated with the findings of khandewal (2002) and Kokate (2005) that the presence of a concoction of different metabolites once gets into the cellular microcosm might hinder cellular growth, through mitodepression and cellular death.

The widespread presence of alkaloids in almost all plant extracts are widely exploited nowadays for cytotoxicity studies as alkaloids are potentially cytotoxic (Patel *et al*., 2012; Silva *et al.*, 2020). Report had shown that bioactive compounds that are anti-carcinogenic in nature may act in a way that could be cytotoxic in cells through mitotic suppression (Akinboro *et al*., 2011). In this present investigation observation of cell death could be accounted and scored in the form of shrinkage in nuclear volume, nuclear fragmentation, sticky nucleus, two interphasic bridged-nuclei, buddings, double nucleus without separation plates and ultimately karryorrhexis and karyolysis were also noted (Graph: 3). Therefore, apart from MI and CA, cell death or nuclear abnormalities were regarded as end-point cytotoxic marker and designated as “biomarker” of mutagenicity as obtained in the test groups treated with CSTAE. We observed a significant number of cells undergoing apoptosis and with nuclear alterations related to cell death processes in the *A. Sativum* and *Lathyrus sativus* L*.,* assays with varying extract concentrations (Figure 2 and 3, Graph: 3 ).The nuclear alterations, observed in large number in the meristematic cells of *A. Sativum and Lathyrus sativus* L*.,* are similar to karyorrhexis (It is the manner of destructive fragmentation of the nucleus of dying cells where the chromatin is irregularly distributed throughout the cytoplasm) and karyolysis (Enzymatic dissolution leads to complete suspension of the chromatin in a dying cell. (Sarkar, Saha and Haldar, 2022) just like a supernova explosion where the nucleus can’t control but explodes as seen in dead stars.

These alterations are invariably responsible for the cell to undergo the death process by necrosis or apoptosis, in which the chromatin is irregularly distributed, including the formation of lumps in the nuclear membrane (Tolbert, Shy, Allen, 1992; Thomas *et al.*, 2008; Lima *et al.*, 2016). Pacífico *et al.* (2013) reported the inducement of apoptosis by apolar extracts of *L. nobilis* leaves rich in cytotoxic compounds within the apolar and aqueous extracts. It has been examined that phyto-fractions with high molecular weight compounds could induce ruptures in the DNA double strand producing apoptosis faster in comparison to the fraction with low molecular weight compounds rather inducing anti-mitosis, promoting a stop in the cell cycle in the G1/S phase (Rood *et al.,* 2015). Different phytoextracts prepared from different parts of medicinal plants has been found to be a rich reservoir of active-biomolecules (Allelochemicals) which altogether can lead to osmolyte imbalance cum ROS outburst in plant root cells leading to DNA damage, altering nucleotide bases,

loss of nucleotides, oxidation of deoxyribose sugar residues thus cross-linking and breaking DNA strands (Das and Roychoudhury 2014). Ciniglia *et al*. (2014) reported reduction in DNA integrity in the target plants, as a result of exposure of maize roots to walnut husk washing waters, with DNase activity resulting DNA fragmentation. Juglone treatment in the root tips of lettuce seedlings (Babula *et al*., 2014) induced DNA fragmentation could be observed whereas in soybean *Eucalyptus* tree litter could induce the same response (Abdelmigid and Morsi 2017) which are the direct outcomes of decreased mitotic index in the root meristem cells of the target plants (Babula *et al*., 2014) in addition to cyanamide (Soltys *et al*., 2011, 2012) and umbelliferone (Yan *et al*., 2016). In the present investigation there was the formation of large vacuole with shrinkage of protoplast and multi-fragmented nuclei (Fig Plate 2: N-P; Fig Plate 3L, Graph: 3) in both *Lathyrus* and *Allium* meristems. These observations are similar to the reports of earlier studies where Excessive vacuolization occurring in the root apical meristem cells of radish (*Raphanus sativus*) exposed to coumarin (Aliotta *et al*., 1993), and in the root cap cells after treatment with an extract of cagaita (*Eugenia dysenterica*) (Pereira *et al*., 2017). Interestingly in the root cap and root apical meristem cells in bottle gourd (*Cucurbita ficifolia*) and bean (*Phaseolus vulgaris*) seedlings a cucurbit extract was responsible for formation of large vacuoles (Cruz-Ortega *et al*., 1998).

The GC-MS analysis of CSTAE revealed the presence of some important Amaryllidaceae type alkaloids Hordenine, Ismine, Trisphaeridine, Crinine, Galanthamine, Anhydrolycorine, Assoanine, Galanthine, Incartine, Lycorine and Galwesine out of which the lycorine series (lycorine, Crinine, galanthamine, Galanthine) and Anhydrolycorine, Incartine, which are Narcissus group of alkaloids; have been reported to be of utmost pharmacological importance (Table: 1, 2, Fig: 1). For the first time Yamaguchi and Suda (1952) reported that lycorine at a concentration of $10^{-2}$ M exhibited germination and growth inhibitory effects in *Vicia faba* L., which could be related to several cytological changes, including inhibition of the metaphasic stage and chromosomal aberrations helped to draw the conclusion that the physiological effects of lycorine must have been mediated through its action on cell division. Further studies concluded that even at lower concentrations ($10^{-4}$ to $10^{-6}$ M), lycorine strongly inhibited cell divisions both in liquid yeast cultures and in lettuce pith parenchyma explants (De Leo *et al.,* 1973). Interestingly, De Leo *et al.,* (1973) revealed that in lycorine-treated pea internodes there notable influence via decrease in 3H-uridine and 14C-leucine into RNA and protein, respectively and studies by Hohmann *et al.,* (2002) conclusively revealed that lycorine formed a complex with RNA to impart anti-proliferative effects. Interestingly reports in recent past revealed that lycorine and its analougue pseudolycorine strongly inhibited the peptide bond formation catalyzed by eukaryotic ribosomes during protein synthesis (Jimenez, Sanchez, and Vazquez 1975). Jimenez *et al.* (1976) reported that lycorine lycorine, pseudolycorine and dihydrolycorine were capable of interacting with DNA via interacting/inhibiting with the topoisomerase enzymes, thereby altering the topology of DNA, either by by overwinding or underwinding of DNA template (Barthelmes *et al.*, 2001; Ancuceanu and Istudor, 2004; Casu *et al.*, 2011). In this investigation, Hordenine (an alkaloid prevalently present in germinating barley seeds) has been reported to be present in CSTAE, could be responsible for cellular apoptosis via stimulation of the extrinsic pathway in living mitochondira (Akhil *et al.*, 2020). Ismine alkaloid is reported to be cytotoxic but shows lower DNA-topoisomerase inhibitory activity than lycorine (Nair and Staden, 2021). Crinine and Galanthamine have been reported to be cytotoxic and antifungal in acitivity (Marikova *et al.*, 2019).

It is well documented that lycorine (and its analogues) appeared to be powerful inhibitor of cell growth, cell division, and organogenesis in higher plants, algae, and yeasts, inhibiting the cell cycle during interphase, which seems to be related inhibition of ascorbic acid (L-Asc) biosynthesis inhibiting L-galactono-g-lactone dehydrogenase, (Davey *et al.*, 1998; Gara *et al.*, 1994; Imai *et al.*, 1998;) the terminal enzyme of L-Asc biosynthesis in mitochondrial membrane (Arrigoni *et al.*, 1997; Co´rdoba-Pedregosa *et al.*, 1996; Giudice *et al.*, 1997; Liso *et al.*, 1984; Onofri *et al.*, 1997). In plants, it also inhibits cyanide-insensitive respiration, peroxidase activity, and protein synthesis (Arrigoni, Arrigoni-Liso and

Calabrese, 1976; Liso *et al.*, 1985; Kukhanova, Victorova, and Krayevsky 1983). Apart from direct interactions with DNA topology and RNA metabolism one cannot completely rule out non-specific/indirect effects of these alkaloids as in yeasts, lycorine is able to interact directly with mitochondrial DNA in conjunction with Galanthine which also has a high capacity to inhibit ascorbic acid biosynthesis (Evidente *et al*, 1983) thereby inducing cellular apoptosis as an final outcome of root growth inhibition through mitochondrial dysfunctioning. Therefore in this experimental set up the CSTAE, being a rich reservoir of Lycorine, Crinine, Galanthamine, Galanthine could synergistically altogether brought about the varied levels of cytotoxic and genotoxic responses in *A. Sativum* and *Lathyrus satvius* L., root tip cells, in addition to G1/S/G2 transitory mitoderpression to apoptotic responses after exposure to low to high doses in the (in vivo) experimental set up probably leading to positive ROS outburst, dysfunctioning of cellular enzyme systems by blocking protein synthesis and mitochondrial poisoning vis-a-vis ascorbic acid (L-Asc) biosynthesis blockage as a final outcome as biochemical pathophysiology (Jerald *et al.*, 2021).

*In vitro* measurement of hemolytic activity of different phytoconstituents can serve “Bioassay-based indicator” for cytotoxicity. Performance of different crude extracts during hemolytic assay could be considered as important determinant to deipher whether a drug possessing any antioxidant/pro-oxidant poteintialities and if these bioactivities can be employed in pharmacological therapeutics. It has been suggested that, the potential phytoconstituents present in different extracts in vitro and in vivo reactions might bring out damaging to plant cell chromosomes could also potentially impart genotoxicity for mammalian cells too (Feretti *et al.,* 2007). Apart from direct action on lipid membrane through pore formation via membrane protein dissolution (Adhikari *et al.*, 2007) or ROS –induced indirect dissolution of the lipid bilayer through hydrolyis of 2-acyl ester bonds of 3-sn-phospholipids producing arachidonic acid and lysophospholipids (Adhikari and Karmakar, 2018) as a combined action of all the other active components i.e., flavonoids, tannis, saponins, phenolics etc (apart from isolated alkaloids of CSTAE) cannot be completely ruled out.

Preliminary phytochemical tests also revealed the strong presence of tannins in CSTAE. Functionally tannins react with proteins as multidentate-ligands (active phenolic rings reacting with proteins, forming -stacking with aromatic side-chains of proteins forming hydrophobic interactions by van der Waals interactions), ultimately leading to protein precipitation (Maatsola *et al.*, 2020). Tannins including tannic acid (water soluble complex polyphenols) have been reported to be stimulators of apoptois via mitochondrial depolarization (Chen *et al.*, 2009), upregulating apoptosis-inducing factors (Chen *et al.*, 2009, Sun *et al.*, 2012), DNA fragmentation by activation of caspases (Labieniec and Gabryelak, 2006).

## CONCLUSION

Finally, a conclusion could be drawn that that CSTAE when applied in high doses (40, 30 and 20 mg/ml) shows cytotoxic and genotoxic activity in germinating root tip cells of Allium cepa and Lathyrus sativus L., In these experimental set up CSTAE crude extract has been assayed as exploration of crude extracts is appropriate because in practice crude *Cascabella* fruits are consumed during suicidal attempts as a functional source of poison and different parts of *Cascabella thevetia* have been exploited as crude extracts to uncover the pharmacological properties per say. However, working with crude extracts also means unearthing the potency of complex mixtures/consortium of biologically active compounds synergistically imparting multi-cascade toxic responses. Some of these compounds can be cytotoxic and/or genotoxic owing to pro-oxidant potency whereas some could impart cytoprotective/ antigenotoxic actions through antioxidant properties. CSTAE is a rich reservoir of pharmacologically rich and varied phyto-components like alkaloids, tannins, saponins, phenolics etc., which must be explored as potential bioactive-therapeutic agents for future drug development with receptor-kinetics studies coupled with bioactivity guided purification and bioassays employing structure-activity relationship studies (SAR) with different biomarkers to draw a conclusive evidence-based pharmaco-mapping although it can cause serious problems and damage on cells when used improperly as opined by Celik and Aslant¨urk, (2010)

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## AUTHORS' CONTRIBUTIONS:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by D.A, the GC-MS analysis and Phytochemical Studies were performed by T.G. and cytoxicity studies involving *Allium sativum* and *Lathyrus sativus* were performed by R.G. were performed manually by S.D. in the laboraty. The first draft of the manuscript was written by D.A. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

## CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

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