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EVALUATION OF PHYTOTOXIC AND CYTOGENOTOXIC POTENTIALS OF LEAF AQUEOUS EXTRACT OF *AMPELOCISSUS LATIFOLIA* (ROXB.) PLANCH. IN RELATION TO ITS TOTAL POLYPHENOL CONTENT

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ABSTRACT

Ampelocissus latifolia (Roxb.) Planch. is very commonly used in traditional medicine to treat a variety of pathological conditions. The present study aims to explore phytotoxic and cytogenotoxic potentials of leaf aqueous extract of *A. latifolia* (LAEAL). Phytotoxic activity of LAEAL was investigated by analysing wheat (*Triticum aestivum)* root morphological changes and fluorescence patterns of ethidium bromide and acridine orange. Tannic acid was used as positive control for fluorescence microscopic analysis. Cytogenotoxic effects of LAEAL were analysed using light and fluorescence microscope on onion (*Allium cepa)* root tip cells. Germinating wheat seeds treated with LAEAL at concentrations 0.5, 2, and 4 mg/mL for 96 h showed root growth retardation respectively as 51, 59 and 80%. Data indicate that LAEAL treated roots were swelled and the number of root hairs reduced in 48 h as compared to untreated controls. 24 h aged onion roots were treated with LAEAL (0.5 and 2 mg/mL), root tips were fixed at 2- 24 h and different cytogenotoxic effects were analysed from the squashed root tip cells. LAEAL induced dose dependent decrease in mitotic index ($p<0.001$) and increased frequencies of different types of chromosomal and cytological changes that may be due to the presence of high percentage (21.03±0.9%, tannic acid equivalent) of polyphenols. This study reveals that the leaf aqueous extract of *A. latifolia* exerts significant phytotoxic and cytogenotoxic effects and therefore this plant should be used in traditional practice with caution.

KEY WORDS: **Polyphenols, *Ampelocissus latifolia*, phytotoxicity, genotoxicity, tannic acid.**

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INTRODUCTION

Plant products are used as a source of medicines in traditional practice for the treatment of various ailments [1]. Crude plant extracts in the form of decoction, infusion or tincture are traditionally used for the treatment of several diseases. Plants synthesize secondary metabolites which have defensive roles against insects, herbivores, infections etc. Recent studies have shown that many plants used in traditional medicines or as food may also have cytogenotoxic effects [2, 3]. This raises serious concern about the potential toxic hazards due to extensive and long term use of these types of plants. So an assessment of their cytoxicity and genotoxicity are compulsory to ensure safe and healthy use of medicinal plants [4]. The phytotoxic and cytogenotoxic effects of plant extracts can be analysed using apical root meristem cells of plants at the level of root morphology and alterations in cellular and chromosomal morphology. *Allium cepa* genetic material has been widely exploited since A. Levan first introduced it as a test system and showed that colchicine could cause spindle disturbances and polyploidy in *Allium cepa* root meristem cells. Later he also demonstrated that various inorganic salt solutions induced different kinds of chromosome aberrations in *A. cepa* root cells [5]. Since then, many new mutagenicity assays using microorganisms, mammalian cells and other biological systems have been developed, but plant tests are still used routinely for genotoxicity testing all over the world [6]. *Allium cepa* root tips are suitable test system for estimating harmful effects of chemicals on biological materials [7, 8]. The toxicity of the toxicants can be observed by easier and the cheapest root tip assay [9 - 11]. Fiskesjo reported that *Allium* test has been found to have a high correlation with other test system (MIT-217 cell test with mice or rats *in vivo*) and could be used as an alternative to laboratory animal in toxicological research [7]. The genus *Ampelocissus* (Family: Vitaceae) is used in traditional medicines for the treatment of a variety of disorders. *A. latifolia* is native herb to Indian subcontinent. The root decoction of this herb is used to treat dental troubles, ulcers, dysentery [12, 13], gout, fractured bone, dyspepsia, indigestion and tuberculosis [14, 15]. It is used as an antidote for snake bite, applied on wounds, abscess and for easy labor and delivery of a baby [16]. Fresh stem node paste is applied on swelled joints of cattle [17]. Phillippine *Ampelocissus* possess various phytochemicals like, alkaloids, fixed oils and fats, flavonoids, saponins, tannins, carbohydrates, glycosides etc. and acetogenins like 22-epicalmistrin, uvaribonin and chalcone were also isolated from the root [18]. Recently the anti-inflammatory, antibacterial and antioxidant activities of this plant have been reported [19-21]. However, no experimental evidences are available regarding its cytogenotoxic effects. *A. cepa* and *T. aestivum* root apical meristem cells are considered as the best models as they exhibit high sensitivity to exogeneous agents and used to study adverse effects of various substances like drugs, pollutants, chemicals by analysing their effects on root growth inhibition and altered chromosome structures [22]. Moreover, plant based cyto-genotoxicity assay enables us to correlate the toxicity of toxicants to those obtained using mammalian models [23].Therefore in the present study cytogenotoxic and phytotoxic potentials of leaf aqueous extract of *A. latifolia* (LAEAL) were studied on wheat and onion root tip cells to assess whether it has any adverse effects on root growth retardation, mitotic index depression and chromosomal aberrations. Moreover phytotoxic potentials of LAEAL were analysed using fluorescence microscope and correlated with its Polyphenol content where tannic acid was used as positive control.

MATERIALS AND METHODS

Plant product collection, storage and extract preparation

Fresh leaves of *A. latifolia* were collected from Burdwan University campus, West Bengal, India in August 2011. This plant species was taxonomically identified by Dr. Ambarish Mukherjee (Taxonomist), Professor, Department of Botany, the University of Burdwan. The voucher specimens (No.BUGBAC012) are maintained in the department for future reference. Collected leaves were washed in tap water, shade dried,

directly crushed into small pieces and followed to pulverize using an electric grinder (Philips Mixer Grinder HL1605). Ground powder was stored in air tight container. 20 g of dried powdered plant material was extracted in 400 ml of distilled water for 6 h at slow heat (50 °C) in water bath. At the end of 6 h extract was filtered through No. 1 Whatman® filter paper and stored at -20 °C for further use. For determining the extract value (29.97±1.3 %) and concentration, few ml of extract was evaporated to dryness with hot air oven.

Experimental plants

Wheat (*Triticum aestivum*) seedlings and onion (*Allium cepa*) roots were used as experimental plant models. Wheat seedlings were used for root growth retardation and phytotoxic assay. Wheat and onion roots were used for fluorescence microscopic detection of phytotoxicity. Onion root apical meristem cells were used for microscopic analysis of cytogenotoxicity.

Triticum aestivum culture and Phytotoxicity tests

The phytotoxic effect of LAEAL was evaluated by examining wheat root growth retardation pattern, studying the characteristic root and root hair morphological changes and fluorescence microscopic analysis of cytotoxicity using acridine orange and ethidium bromide double staining method on LAEAL treated wheat root tips and compared with the untreated controls which were maintained in distilled water. Simultaneously tannic acid (poly phenolic compound) was used as positive control for this study. Wheat seeds were surface sterilized with 1% Sodium hypochlorite following standard protocols as earlier described in detailed [24, 25]. For morphometric analysis of growth retardation, wheat seeds were exposed to different concentrations (0.5, 2 and 4 mg/mL) of LAEAL at the beginning of the experimental set up. Wheat seeds were soaked in LAEAL in Petri dish and covered with another Petri dish and kept in the dark at 25°C temperature for germination. Root lengths were measured at 24, 48 and 96 h. Three replica of each with 10 seeds were prepared for each treatment and untreated controls. Only distilled water was used as a culture medium for untreated control seedlings. For LAEAL induced morphological change analysis, wheat roots (24 h aged) were exposed with LAEAL (0.5, 2 and 4 mg/mL) for 48 h and morphological changes were recorded and photomicrographs were taken.

Ethidium bromide and acridine orange staining to detect phytotoxicity with fluorescence microscope

Wheat and onion roots were analysed with ethidium bromide and acridine orange double staining procedure for fluorescence microscopic detection of phytotoxicity. For LAEAL induced cytological change analysis, wheat and onion roots (24 h aged) were exposed with LAEAL (0.5 - 4 mg/mL) for 24 and 48 h. Simultaneously tannic acid (0.8 mg/mL) treatment was given for 24 h to wheat seedlings and considered as positive control. Equal concentration (0.01%) of ethidium bromide and acridine orange solutions were mixed just before the use. Treated and untreated roots were stained for 2 minutes, washed in distilled water thoroughly, squashed on glass slide under cover slip and the colour patterns were observed with Leica fluorescence microscope. Acridine orange can penetrate in both the living and dead cells while ethidium bromide penetrates only in dead cells and they stain differentially. Blue filter excitation on living cells stained with acridine orange give green colour, early apoptotic cells allowing limited penetration of ethidium bromide possess green to yellowish nuclei with perinuclear chromatin condensation while late apoptotic cells coloured in dark red possess fragmented or condensed chromatin.

Cyto-genotoxic analysis with Allium cepa root apical meristem cells

The cytogenotoxic activities of LAEAL were screened using onion (*Allium cepa)* root apical meristem cells. Onion bulbs were allowed to sprout in distilled water for 48 h maintaining at 25°C. The newly emerged equal sized onion roots were treated with two concentrations (0.5 and 2 mg/mL) of LAEAL for 2, 4, 6 and 24 h and simultaneously the control group was maintained in distilled water. For each dose and treatment hours at least 3 onion bulbs were used. The treated and untreated roots

were fixed in aceto-methanol (3 parts methanol: 1 part glacial acetic acid) for 24 h, washed with distilled water and hydrolyzed in 1N HCl at 60°C for 5 minutes. For each treatment at least six slides were prepared using aceto-orcein (2%) squash technique [24-26]. Slides were studied under bright field light microscopy with 40X objective lens and minimum 2400 cells were scored. Mitotic indices and frequencies of chromosomal and nuclear abnormalities were scored.

Estimation of total phenol content

Total phenol content of LAEAL was measured by using Folin-Ciocalteu reagent [25, 27]. 10µL of each sample was taken in the respective test tubes and the volume was made up to 1 mL by adding distilled water. Then 0.5 mL of Folin-Ciocalteu (1N) reagent was added and mixed properly. After that 2.5 mL 20% sodium carbonate was added to each sample tube and the test tubes were kept in the dark for 40 min. The optical density was measured at 725 nm using Spectrophotometer (UV-1800 Series, Shimadzu, Japan). Total phenol content was estimated using the tannic acid, a specific type of commercial polyphenol, standard curve. Standard tannic acid solution (0.5 mg/mL) was prepared by dissolving 5 mg tannic acid in 10 mL distilled water. From this stock solution different standard concentrations (2.5-25µg/mL) were prepared by serial dilution method.

Scoring and statistical analysis

The difference between the control and treated groups for the root length was analyzed with student's t test. The statistical significance of the difference between the control and treated groups for MI, cell abnormality percentage and abnormality per cell were analysed using a 2X2 contingency χ2-test. Differences between corresponding controls and exposure treatments were considered statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$. Patterns of cell cycle kinetics were determined by scoring of the number of cells in dividing phase / Total number of cells scored X 100.

RESULTS

Wheat root growth retardation

LAEAL showed dose dependent growth retardation effects on wheat roots. At 96 h of LAEAL treatment the root growth inhibition was calculated as 51, 59 and 80% respectively for the concentrations of 0.5, 2 and 4 mg/mL and IC_{50} value was calculated as 1.8 mg/mL (Fig.1).

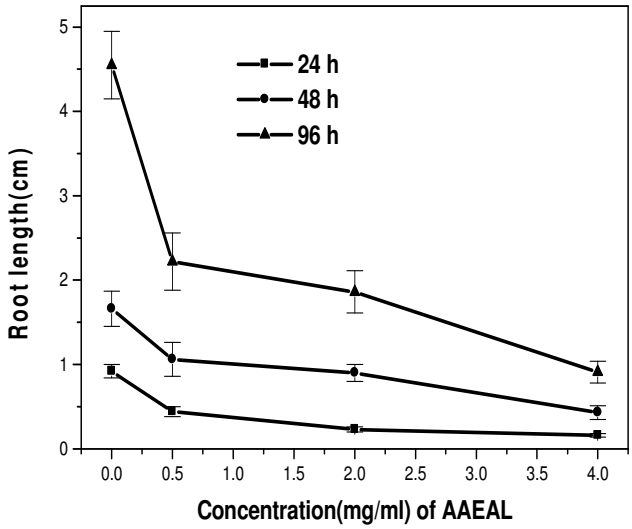

Figure 1

Showing dose dependent root growth retardation effects of LAEAL on wheat roots.Each data point is the mean root length of 30 roots, triplicate set of 10 roots.

Wheat root morphology characterization

Morphological change is an important parameter to assess phytotoxic activity of the test materials in wheat roots [24, 25]. Here, all the used concentrations of LAEAL caused altered root morphology. Gradual swelling of roots occurred with number of root hairs diminished in a dose dependent manner. Colour of the roots was normal in control group, where as the colour changed to yellowish brown after LAEAL application (Fig. 2)

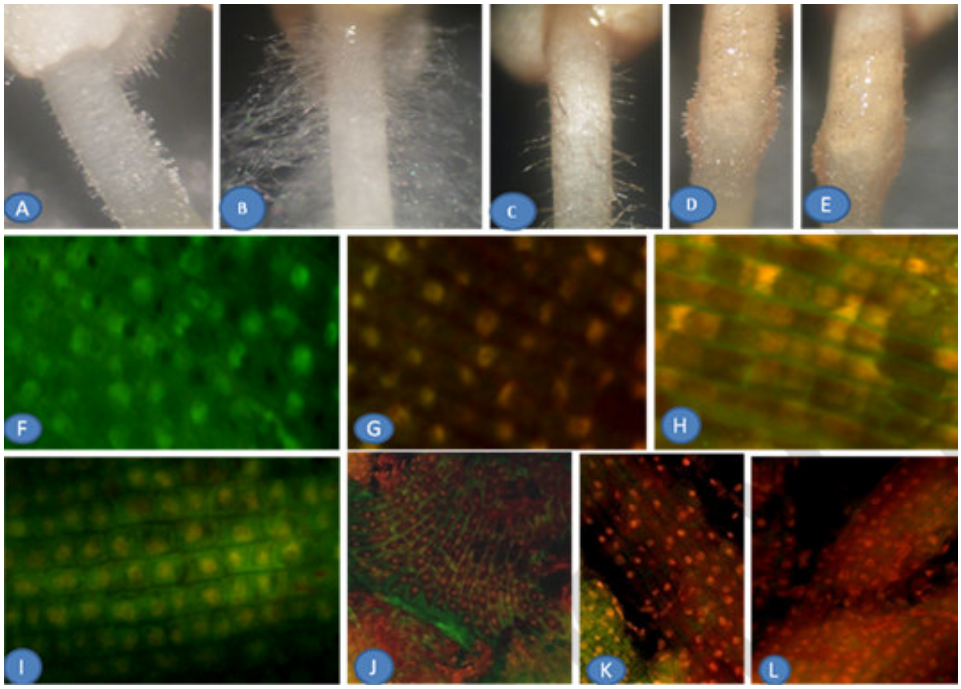

Figure 2

Photographs (C-E) showing LAEAL induced wheat root morphology changes where dose dependent reduction in root length, reduced root hair number and length and increased root swelling were observed. A; untreated root before treatment at 24 h after sprouting, B; untreated root at 72 h, C, D and E; after 48 h of LAEAL treatment respectively at concentrations 0.5, 2 and 4mg/mL. Photographs were obtained with stereomicroscope using 4X objective lens. Photomicrographs G and H; 2 and 4 mg/mL LAEAL respectively for 24 h and I; tannic acid 0.8 mg/mL treatment for 24 h, and J-L; 0.5, 2 and 4 mg/mL LAEAL treatment respectively for 48 h where damaged cells showed ethidium bromide fluorescence (red) while untreated controls (F) showed acridine orange fluorescence (green).

Phytotoxicity test with fluorescence microscope

For fluorescence microscopic detection of phytotoxicity, wheat and onion roots were treated with different concentrations of LAEAL. Ethidium bromide and acridine orange double staining analysis indicated dose dependent phytotoxicity and characteristic colour patterns (Fig. 2 and 3), where damaged cells showed ethidium bromide fluorescence (red) and untreated controls showed the acridine orange fluorescence (green). Tannic acid, used as positive control for polyphenols also showed ethidium bromide fluorescence in wheat roots at 24 h indicating its phytotoxicity.

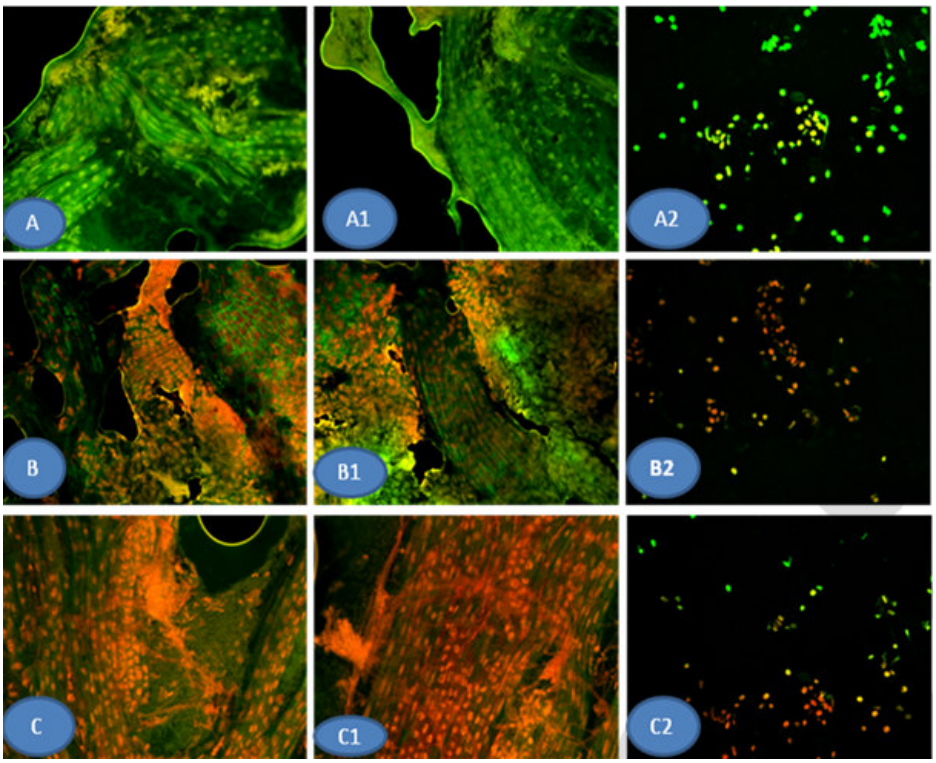

Figure 3

Photomicrographs (B, B1, B2 and C, C1, C2) showing respectively for 0.5 and 2 mg/mL for 48 h LAEAL induced phytotoxicity in onion root tip cells where damaged cells showing ethidium bromide fluorescence (red) while untreated controls showing acridine orange fluorescence (green) (A, A1 and A2). Photograph A2, B2 and C2 were taken from completely smashed area of squashed root tips.

Cyto-genotoxic Analysis

Cytogenetic analysis was performed to investigate the possible mechanisms involved in root growth inhibition in onion root tip cells after the extract exposure. LAEAL induced cytological changes and chromosomal aberrations in LAEAL treated root apical meristem cells. Data show treatment of LAEAL (0.5 and 2 mg/mL) to onion root tip cells produced significant ($p<0.001$) reductions in the mitotic indices in apical meristem cells. Moreover, chromosomal and nuclear abnormalities such as the presence of c-metaphase, sticky and clumped chromosome, delayed and laggered chromosome, anaphase and telophase bridge, vagrant chromosome, chromosome loss, micronucleus, large polytene chromosome, interphase nucleus condensation, binucleate cells etc. were observed (Fig. 4). The percentage of aberrant cells frequency increased significantly at 0.5 and 2 mg/mL concentrations of LAEAL compared to the untreated controls in all the treated hours (Table1).

Table1

Data showing LAEAL induced cytogenotoxic stress in onion root apical meristem cells

Conc. (mg/m)	[h]	T.C.	T.Abn. C. (%)	T.I.C.	Abn.I.C. (%)	T.D.C. (MI %)	Abn.D.C. (%)	T.Abn.	Abn./DC.
0.0	[2]	3326	77 (2.3)	3171	27(0.9)	155 (4.7)	52(33.5)	54	0.35
0.5		3000	112 (3.7)[b]	2864	47(1.6)[b]	136 (4.5)	65(47.8)	73	0.54
2.0		6593	670(10.2)[a]	6470	587(9.1)[a]	123 (1.9)[a]	83(67.5)[b]	99[a]	0.80
0.0	[4]	3717	86 (2.3)	3545	48(1.4)	172 (4.6)	38(22.1)	39	0.23
0.5		4937	245 (5.0)	4815	168(3.5)[a]	122 (2.5)[a]	77(63.1)[a]	100[a]	0.82
2.0		6265	815 (13.0)[a]	6215	776(12.5)[a]	50 (0.8)[a]	39(78.0)[a]	48[a]	0.96
0.0	[6]	4093	88 (2.2)	3891	38(1.0)	202 (4.9)	50 (24.8)	50	0.25
0.5		2939	200 (6.8)[c]	2847	135(4.7)[a]	92 (3.1)[a]	65(70.7)[a]	79[a]	0.86
2.0		3235	517 (16)[a]	3222	508(15.8)[a]	13 (0.4)[a]	09(69.2)[a]	12[a]	0.92
0.0	[24]	2400	58(2.4)	2258	32(1.42)	142(5.9)	26(18.3)	28	0.20
0.5		5888	371(6.3)[a]	5749	280(4.9)[a]	139(2.4)[a]	91(65.5)[a]	120[a]	0.86
2.0		4208	756(18.0)[a]	4208	756(18.0)[a]	-	-	-	-

[a] Significant at p<0.001, [b] at p<0.01 [c] at p<0.05 as compared to their respective control with 2x2 contingency χ2 -test (d.f. = 1). h; hours, T.; Total, C.; Cell, Total Cells scored, TAbn.C.; Total no. of abnormal cells, T.I.C.; Total number of interphase cells, Abn.I.C.; Abnormal interphase cells, T.D.C.; Total no. of dividing cells, Abn.D.C.; Abnormal dividing cells, T.Abn.; Total no. abnormality scored, Abn./DC.; Abnormality per dividing cell.

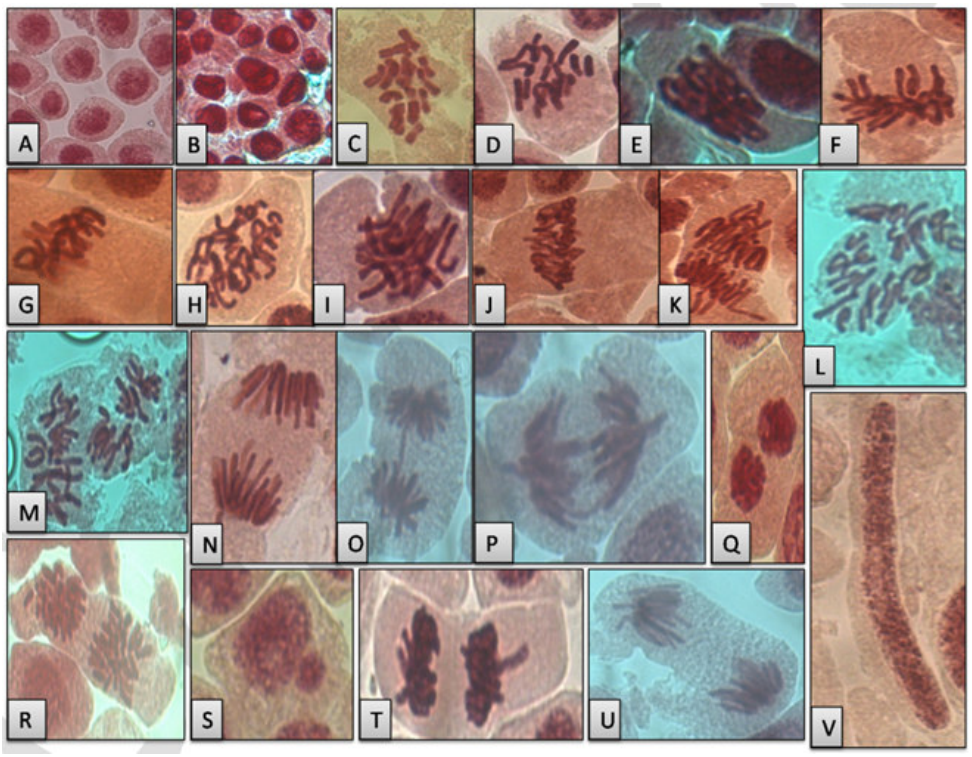

Figure 4

Chromosomal abnormalities observed in onion root apical meristem cells after different hours of LAEAL treatment at conc. of 0.5 and 2 mg/mL. A-morphology of untreated nucleus, B- nuclear condensation after treatment with LAEAL, C and D –c metaphase, E- sticky or clumped chromosome at metaphase, F- chromosome loss at metaphase, G- ring formation, H- ball metaphase, I – unorganized chromosomes at metaphase, J-chromosome laggards at anaphase, K, P- anaphase stage with both vagrant chromosome and bridge formation, L, M- unorganized chromosomes at anaphase, N- anaphase with vagrant chromosome, O- telophase bridge, Q- binucleate cell, R- star like anaphase, S- micronucleus, T, U- telophase with vagrant chromosome, V- giant chromosome.

Total phenol content

LAEAL contains 21.03±0.9 % total phenols as tannic acid equivalent.

DISCUSSION

Ampelocissus latifolia is being used for the treatment of a variety of disorders [28- 32]. The work presented herein focuses on the phytotoxic and cytogenotoxic effects of LAEAL on wheat and onion root apical meristem cells. Toxicity of LAEAL was investigated through morphometric bioassays considering the root length and root morphology changes of wheat seedlings, and cytogenetic analysis of onion root apical meristem cells. Moreover, fluorescence microscopic study was performed on wheat and onion root apical meristem cells. In the initial experiments, a wide range (0.25–6 mg/mL) of LAEAL concentrations were used for morphometric bioassays on green gram seedlings and onion roots (data not shown here), and finally 0.5 to 4 mg/mL concentrations were selected for microscopic analysis of cytotoxicity on root tip cells. The present study clearly indicates phytotoxic and cyto-genotoxic effects of LAEAL. LAEAL treatment on wheat roots at concentrations 0.5, 2 and 4 mg/mL respectively could induce growth retardation 51, 59 and 80%. Thus LAEAL treatment could significantly ($p<0.001$) reduce the root length of wheat seedlings in a dose dependent manner (Fig 1). A number of earlier studies have also shown that the percentage of growth retardation of roots increases with increasing extract concentrations [24, 25]. Plant derived polyphenols are the secondary metabolites and are recognised as allelochemicals. These are the most abundant substances that affect seedling growth and cell division [33]. Recently, leaf aqueous extracts of *Clerodendrum viscosum* and *Synedrella nodiflora* also shown to have antiproliferative effects on root apical meristem cells [24, 25]. In the present study, the LAEAL exerted pronounced mitodepressive effects on onion root meristem cells at all the applied concentrations. The LAEAL treatment on *A. cepa* root at a concentration of 2 mg/mL for 6 h led to reduction of Mitotic index (MI) by 91.84%. The extract also caused nuclear condensation associated with apoptosis in plant cells (Fig. 4). Similar findings were also reported with other plant extracts [34, 35]. The results herein suggest that the LAEAL has phytotoxic effects on root apical meristem cells of *A. cepa*. In LAEAL treated onion apical meristem cells, a variety of chromosomal abnormalities were induced along with significant decrease in mitotic index in a dose and time dependent manner. MI measures the proportion of cells in the dividing phase of the cell cycle and decrease in MI is associated with cytogenotoxicity of the agents [36, 37]. Reduction in mitotic index can be explained as being due to delay in the onset of prophase, or the arrest of one or more mitotic phases or the slowdown of the rate of cellular progression through division cycle [38]. Some of the efficient anticancer and anti-neoplastic agents exert their effect through the cell cycle progression machinery [39]. Mitostatic activity of some plant extracts, including the ability to block DNA synthesis was also reported earlier [35, 40]. The presence of various chromosomal abnormalities like chromosome bridges, c-metaphase, ball metaphase, binucleate cells, lagging chromosomes at anaphase, star like anaphase, unequal grouping at metaphase, vagrant chromosome, sticky and clumped chromosomes reinforces the hypothesis of toxic effects of the LAEAL (Table 1). The presence of chromosomal bridges and chromosomal breaks indicate clastogenic effect, while chromosome loss, delayed alignment, sticky chromosome and c-metaphase results from aneugenic effects [37]. Chromosomal stickiness results due to agglomeration [41]. Presence of chromosomes with sticky or clumped metaphases, suggests that the extract interferes with chromatin organization through an imbalance in the proportion of structural proteins such as histones [10]. The formation of chromosomal breaks and ball metaphase indicates its effect on mitotic spindles. Mitotic spindle disturbance also causes separation of daughter chromosomes parallel to the equator instead of towards the pole [42]. The presence of lagging chromosomes may be attributed to the delayed terminalization, stickiness of chromosome ends or failure of chromosomal movement [43]. Inhibition of cytokinesis is responsible for binucleate cell formation [44]. Formation of anaphase and telophase bridge occurs due to chromosome breakage, stickiness and breakage-fusion-bridge cycles [45]. An important observation was that the LAEAL exerted strong toxicity in *Allium cepa*,

as evidenced by the frequent occurrence of sticky metaphase, leading to cellular death. Percentage of number of abnormalities per cell was also increased in both the treatment concentrations in a time dependent manner, excepting the case for 2mg/mL, 6 h and 12 h. It can be explained by the fact that longer time of exposure with the highest concentration (2mg/mL) drastically diminished the existence of dividing cells as the cells became arrested at interphase stage, most probably due to interphase chromatin condensation, which was evidenced by the presence of highly condensed state of nucleus as compared to the control (Fig 4). The results of the present investigation suggest the potential use of *A. latifolia* as a therapeutic agent. Its effect on cell division phases may be attributed to its use as an antitumor or anticancer agent. We used crude extract for this study. Crude extract is a complex mixture of phytoconstituents and some of these may have cytogenotoxic effects while others may have protecting effects. Studying with crude extract means acquiring total idea regarding its potentiality as a therapeutic agent. The LAEAL induced chromosomal abnormalities directly correlates its cytogenotoxicity. It is well-known that the phenolic compounds are the most abundant substances which can inhibit root growth and cell division, thus causing growth retardation and finally cell death [46, 47]. Our study reveals high amount of phenolic content (21.03±0.9% of the extract) in LAEAL. The therapeutic potentials of medicinal plants lie in their phytochemical ingredients [48]. The higher quantities of total phenolic content in LAEAL and the phenomenon of interphase chromatin condensation, c-metaphase, and a variety of chromosomal aberrations in root apical meristem cells were possibly be the reason behind the cytotoxicity. To ascertain the toxic effects of polyphenols of LAEAL, fluorescence microscopic analysis was performed. Tannic acid showed similar damage to wheat roots and the fluorescence patterns of ethidium bromide and acridine orange (Fig. 2). Therefore, the toxicity of LAEAL may due to the presence of the higher percentage of polyphenolics. In conclusion we remark that although *A. latifolia* may be very important and extensively used in folk medicine its indiscriminate use may be dangerous and therefore proper dose must be prescribed as cytogenotoxic potentials are explored here. Further studies are needed to find out its bioactive principles responsible for cytogenotoxic property and to trace out the underlying mechanism of its activity.

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REFERENCES

1. Bako SP, Bakfur MJ, John and Bala EI, Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. Int J Bot, 1(2), 147-150, (2005).
2. Higashimoto M, Purintrapiban J, Kataoka K, Mutagenicity and antimutagenicity of extracts of three spices and a medicinal plant in Thailand. Mutat Res, 303 (3), 35–142, (1993).
3. Askin CT, Aslanturk S, Cytogenotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. Biologia, 62(3), 292–296, (2007).
4. Lubini G, Fachinetto JM, Laughinghouse HD, Paranhos JT, Silva ACF & Tedesco SB, Extracts affecting mitotic division in root-tip meristematic cells. Biologia, 63, 647–651, (2008).
5. Levan A, The effect of colchicine on root mitosis in *Allium*. Hereditas, 24, 471-486, (1938).
6. Grant WF, Higher plant assays for the detection of chromosomal aberrations and gene mutations—a brief historical background on their use for screening and monitoring environmental chemicals. Mutat Res, 426, 107–112, (1999).
7. Fiskesjo G, The Allium tests as a standard in environmental monitoring. Hereditas, 102, 99-112, (1985).

8. Rank J and Nielsen MH, Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telophase chromosome aberration assay. Mutat Res Genet Toxicol Environ Mutagen, 418,113-119, (1998).
9. Sobita K and Bhagirath T, Effects of some medicinal plant extracts on *Vicia faba* root tip chromosomes. Caryologia, 58, 255–261, (2005).
10. Kuras M, Nowakowsaka J, Sliwinska E, Pilarski R, Ilasz R, Tykarska T, Zobel A, Gulewicz K, Changes in chromosome structure, mitotic activity and nuclear DNA content from cells of Allium Test induced by bark water extract of *Uncaria tomentosa* (Willd.) DC. J Ethnopharmacol, 107, 211–221, (2006).
11. Sousa SM and Viccini LF, Cytogenotoxic and genotoxic activity of *Achillea millefolium* aqueous extracts. Braz J Pharmacog, 21, 98–104, (2011).
12. Mishra R and Billore KV, Some Ethnobotanical lores from Banswara District. Nagarjun, 26(10), 229-231, (1983).
13. Patil KJ and Pati SV, Biodiversity of Vulnerable and Endangered Plants from Jalgaon District of North Maharashtra. Asian J Pharmacy and Life Sci, 2(2), 144-150, (2012).
14. Swarnkar S and Katewa SS, Ethnobotanical observation on Tuberous Plants from Tribal Area of Rajasthan (India). Ethnobot Leaflets, 12, 647-666, (2008).
15. Prusti AB and Behera KK, Ethnobotanical exploration of Malkangiri district of Orissa, India. Ethnobot Leaflets, 11, 122-140, (2007).
16. Patil MV and Patil DA, Ethnomedicinal practices of Nasik District, Maharashtra. Int J Traditional Knowledge, 4(3), 287-290, (2005).
17. Patel RS, Kanjaria KV, Patel KC, Investigations on climber resources used by tribal inhabitants of Ambaji forest of Banaskantha District (North Gujarat). Life Sci Leaflets, 10, 251-257, (2010).
18. Pettit GR, Mukku VJ, Craqq G, Herald, DL, Knight JC, Herald CL, Antineoplastic agents.558. *Ampelocissus sp*. cancer cell growth inhibitory constituents. J Nat Prod, 71(1), 130-133, (2008).
19. Tamilarashi CT, Subasini U, Kavimani S, Jaykar B, Phytochemical and pharmacological evaluation of *Ampelocissus latifolia*. Anc Sci of Life, 20(1), 14-18, (2000).
20. Choudhury S, Chowdhury HR, Mandal S, Pharmacognostic studies of *Ampelocissus latifolia* (Roxb.) Planch. an important ethnomedicinal plant. Intern J Curr Res, 5(3), 643-648, (2013).
21. Pednekar PA and Raman B, Antimicrobial and antioxidant potential with FTIR analysis of *Ampelocissus latifolia* (Roxb.) Planch. leaves. Asi J Pharmac and Clin Res, 6(1), 157-162, (2013).
22. Fiskesjo G, *Allium test*. In: Ottare S, Atterwill CK (eds) Methods in Molecular, Biology Humana Press, Inc Totowa, NJ USA, (1996).
23. Celik TA and Aslanturk OS, Cytogenotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. Biologia, 62, 292–296, (2007).
24. Ray S, Kundu LM, Goswami S, Roy GC, Chatterjee S, Dutta S, Chaudhuri A, Chakrabarti CS, Metaphase arrest and delay in cell cycle kinetics of root apical meristems and mouse bone marrow cells treated with leaf aqueous extract of *Clerodendrum viscosum* Vent. Cell Prolif, 46, 109–117, (2013a).
25. Ray S, Chatterjee S, Chakrabarti CS, Antiproliferative activity of allelochemicals present in aqueous extract of *Synedrella nodiflora* (L.) Gaertn. in apical meristems and wistar rat bone marrow cells. Iosr J Pharm, 3(2), 1-10, (2013b).
26. Murthy GS, Francis TP, Singh CR, Nagendra HG, Naik C, An assay for screening anti-mitotic activity of herbal extracts. Curr Sci, 100(9), 1399-1404, (2011).
27. Dutta S and Ray S, Evaluation of antioxidant potentials of leaf aqueous and methanolic extracts of *Calophyllum inophyllum* in relation to total phenol and flavonoid contents. Int J Pharm Bio Sci, 5(3), 441 – 450, (2014 July).
28. Ashourand SA and Abdou RF, The action of igran, topograd and eptam herbicides on germination, seedling growth and mitotic behaviour of faba

bean (*Vicia faba* L.). FABIS. Newsletter, 26, 10–14, (1990).
29. Amon A, The spindle checkpoint. Curr Opin Genet Dev, 9, 69–75, (1999).
30. Burke DJ, Complexity in the spindle checkpoint. Curr Opin Genet Dev, 10, 26–31, (2000).
31. Camparoto ML, Teixeira RO, Mantovani MS, Vicentini VEP, Effects of *Maytenus ilicifolia* Mart. And *Bauhinia candicans* Benth. infusions on onion root-tip and rat bone-marrow cells. Genet Mol Biol, 25, 85–89, (2002).
32. Angayarkanni J, Ramkumar KM, Poornima T, Priyadarshini U, Cytotoxic activity of *paeoniifolius* tuber extracts *in vitro*. Am Eurasian J Agric Environ Sci, 2, 395–398, (2007).
33. Lodhi MAK, Role of allelopathy as expressed by dominating trees in a low land forest in controlling the productivity and pattern of herbaceous growth. J Bot, 63, 1–8, (1976).
34. Solomon M, Belenghi B, Delledonne M, Menachem E, Levine A, The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in plants. Plant Cell, 11, 431–443, (1999).
35. Akinboro A and Bakare AA, Cytogenotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa*. Linn. J Ethnopharmacol, 112(3), 470–475, (2007).
36. Rojas E, Herrera LA, Sordo M, Mitotic index and cell proliferation kinetics for identification of antineoplastic activity. Anti-Cancer Drugs, 4(6), 637–640, (1993).
37. Leme DM and Marin-Morales MA, *Allium cepa* test and environmental monitoring: A review on its application. Mutat Res, 682, 71–81, (2009).
38. Christopher HB and Kapoor MB, The cytogenetic effects of sodium salicylate on the root meristem cells of *Allium sativa* L. Cytologia, 54, 203-209, (1988).
39. Salmon ED, Mickseel M, Hays T, Rapid rate of tubulin dissociation from microtubule in the mitotic spindle *in vivo* measured by blocking polymerization with colchicine. J Cell Biol, 99, 1066–1075, (1984).
40. Mercykutty VC and Stephen J, Adriamycin induced genetic toxicity as demonstrated by *Allium cepa* test. Cytologia, 45(4), 769–777, (1980).
41. Babich H, Segall MA, Fox KD, The *Allium* test— a simple, eukaryote genotoxicity assay. Am Biol Teacher, 59(9), 580–583, (1997).
42. Ford JH and Correll AT, Chromosome errors at mitotic anaphase. Genome, 35, 702–705, (1992).
43. Permjit K and Grover IS, Cytological effects of some organophosphorus pesticides. Meiotic effects. Cytologia, 50, 199–211, (1985).
44. Majewska AE, Wolska E, Sliwinska M, Furmanowa N, Urbanska A, Pietrosiuk A, Zobel A, Kuras M, Antimitotic effect, G2/M accumulation, chromosomal and ultrastructure changes in meristematic cells of *Allium cepa* L. root tips treated with the extract from *Rhadiola rosea* roots. Caryologia, 56, 337 – 351, (2003).
45. El-Ghamery AA, El-Kholy MA, Abou El-Yousser MA, Evaluation of cytological effects of Zn^{2+} in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum*. L. Mutat Res, 537, 29–41, (2003).
46. Li ZH, Wang Q, Ruan X, Pan CD, Jiang DA, Phenolics and plant allelopathy. Molecules, 15, 8933–8952, (2010).
47. Rudner AD and Murray AW, The spindle assembly checkpoint. Curr Opin Cell Biol, 8, 773–780, (1996).
48. Phan TT, Wang L, See P, Grayer RJ, Chan SY, Lee ST, Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: implication for cutaneous wound healing. Biol Pharm Bull, 24, 1373–1379, (2001).