



Cytotoxic Effects of *Terminalia Chebula* on Meiotic & Mitotic Chromosomes of *Vicia Faba*

KEYWORDS

Medicinal plant, chromosome, plant extract, anomalies, stickiness, cytotoxicity

Monika Asthana

Department of Biotechnology, School of Life Sciences, Khandari Campus, Dr. Bhim Rao Ambedkar University, Agra-282004, U.P. India

Rakesh Kumar Sarbhoy

Department of Botany, Agra College, Dr. Bhim Rao Ambedkar University, Agra-282004, U.P. India

Surabhi Mahajan

Department of Microbiology, School of Life Sciences, Khandari Campus, Dr. Bhim Rao Ambedkar University, Agra-282004, U.P. India

Avnish Kumar

Department of Biotechnology, School of Life Sciences, Khandari Campus, Dr. Bhim Rao Ambedkar University, Agra-282004, U.P. India

ABSTRACT

There is known curative effect related to heart and digestion disorders by the fruits of *Terminalia chebula* irrespective of rationale dose. Here we showed effect of different concentrations (5, 10, 20, 30, 40, & 50%) of *T. chebula* extracts on meiotic and mitotic chromosomes of *Vicia faba* plant for 4 different time periods. Flower bud treatment method and root tip treatment methods were used for meiotic and mitotic analysis, respectively. Mitotic and meiotic chromosome indicated various abnormalities against higher doses. Cytotoxicity was inferred, Mitotic index of treated cells was significantly different ($P < 0.05$) from control. The percent of abnormalities increase with increasing dose (5-50%) and time (4-24 hours). Stickiness was more frequent than other abnormalities. Higher dose was shown more cytotoxic effect; concluding *Terminalia chebula* extract could be toxic with injudicious dose.

INTRODUCTION

In the present endeavor an effort is needed to study the cytogenetical effects of medicinal plants. Previous studies have revealed that the abnormalities induced by herbal extracts were more or less same type but their frequency varies from plant to plant. Many plant extracts and their active principles have been described and utilized as therapeutic agents. There is considerable interest in determining the risks that these products may pose to health, because many of these plants contain compounds which are known to cause diseases or even death in animals and humans. Thus, an assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants (Surh and Ferguson, 2003). Hence, *Terminalia chebula* were administered in test plant- *Vicia faba*, with a view to access cytotoxic, phytotoxic, clastogenic, teratogenic, turbagenic, properties.

Terminalia chebula is a herbaceous plant belongs to the family Combretaceae of order Rosales. Its common habitat is Southern Asia region. The tree is found in the forests of Northern India, central provinces and Bengal, common in Madras, Mysore and in the Southern parts of the Bombay presidency; it is also found in Burma and Ceylon up to 5,000 ft in the outer Himalayan and up to 6,000 ft in Travancore. The every part of the tree has been used in the preparation of many ayurvedic medicines for household remedy against various human ailments, from antiquity. The fruit Myrobalans are a safe and effective purgative, astringent and alternative. Unripe fruits are more purgative removing bile and phlegm and to adjust bile (Bag et al., 2013) and the ripe are astringent (Lee et al., 2010). Both the fresh and dried fruits of the *T. chebula* are very popular traditional medicines for the treatment of diabetes. It is also reported to have antioxidant and free radical scavenging activities (Cheng et al., 2003).

It also contains astringent principles; tannin (tannic acid) 45 p.c., and a large amount of gallic acid, chebulinic acid which when heated in water splits up into tannic and gallic acids (Inamdar and Rao, 1962).

An assessment of their cytotoxic and mutagenic potential is

necessary to ensure a relatively safe use of medicinal plants (Surh and Ferguson, 2003). It is known that plant cytotoxic bioassays have a good correlation with mammalian cell based assays [21-28]; therefore we studied various dose of *T. chebula*, if have any risk of toxicity. *Vicia faba* L. ($2n = 12$), of the family Fabaceae, have homozygous genotype because of self pollination (Gulfishan et al., 2010). Thus, *Vicia faba* based bioassays have established role to study chromosome anomalies due to their large and visible chromosome (Asthana and Kumar, 2014).

MATERIAL AND METHODS

Collection of samples : The fruits of *Terminalia chebula* were purchased from local market of Agra, India. The same were cross-identified by their vernacular names and later validated at the Department of Botany, School of Life Sciences, Dr. Bhim Rao Ambedkar University, Agra. Voucher specimens (accession number Bot.0001/2012/0008) were deposited for future reference in the herbarium of same department.

Processing of samples: The dried fruits (300 gm) of *Terminalia chebula* (Family: Combretaceae) were washed thoroughly with tap water. After that, fruits were placed in shade to dry for 10-15 days. All dried material was chopped into small fragments. They were then reduced into a fine powder with a kitchen grinder. The powder could then pass through a sieve of pore size 0.5 mm. The part left in sieve was grinded again and again till we get all material in coarse powder.

Preparation of Methanol extraction: Powdered samples (30 gm) were extracted at 65°C with methanol (400 ml) for 72 h (25 cycles) using Soxhlet apparatus to make methanol extract. Finally, the extract was filtered and concentrated in oven at 40°C ± 5°C under atmospheric pressure, to obtain semisolid paste, after drying; they were weighed in order to know the amount of extract of plant sample and percentage yield. The same procedure of extract preparation was repeated with the remaining powder for two times more (Roy et al., 2012). The extract was collected and weighed then diluted with methanol to prepare 5%, 10%, 20%, 30%, 40%, and 50% weight/volume.

For meiotic analysis: Flower bud treatment: Growing plants of *Vicia faba* (35 days old) were sprayed at the flowering time with varying concentration (5, 10, 20, 30, 40 & 50 %) of extract. The spraying of plant was done for two consecutive days. Now the young buds were collected before anthesis at early morning and fixed in acetic alcohol (1:3) mixed with few drops of ferric acetate solution for 6-15 hrs. After fixation the material was washed with distilled water and stored in 70% alcohol. To study meiotic behavior anthers were macerated in one drop of 2% acetocarmine (Haroun and Al Shehri, 2001).

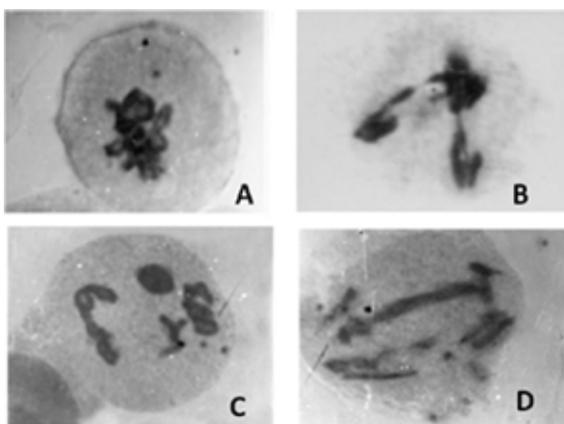
For mitotic analysis: Root tip treatment: To analyze root tip, healthy seeds of *Vicia faba* were germinated on moist filter paper in petri-dishes without soaking in extract solutions. Then germinated seeds having root tips of about 0.5 to 1 cm length were cut from seeds and transferred into varying concentration (5% to 50%) of extract for 4, 8, 12 and 24 hrs duration (Haroun and Al Shehri, 2001). Excised tips were fixed in freshly prepared fixative solution acetic alcohol (1:3) for 24hrs. Feulgen squash technique was used according to Darlington and LaCour (1976) and examined microscopically for mitotic analysis.

Statistical Analysis: The percentage of aberrations at each dose of each pesticide was compared with that of the negative control using the one way ANOVA. One-way ANOVA followed by Turkey's multiple comparisons test was performed using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com to differentiate means [GraphPAD prism ver 6.0]. A dose of extract was considered to be toxic if Turkey's comparison test was significant at $P < 0.05$.

RESULTS

Meiotic study: Varying concentration of *Terminalia chebula* induced various abnormalities viz stickiness, multipolar spindle, multivalent, chromatin bridges, etc (Table 1, Figure 1) in the pollen mother cells of *Vicia faba*. The treated plant exhibit various degrees of abnormalities depends more or less on the concentration applied.

Figure 1: Effect of higher doses of Terminalia chebula extract on meiotic chromosome- A. Diakinesis: Clumping of bivalents, B. Metaphase II: Tripolar showing interconnected bridges C. Diakinesis: Association of bivalents into hexavalents, quadrivalents and bivalents; D Late sticky anaphase with thin chromatin bridge

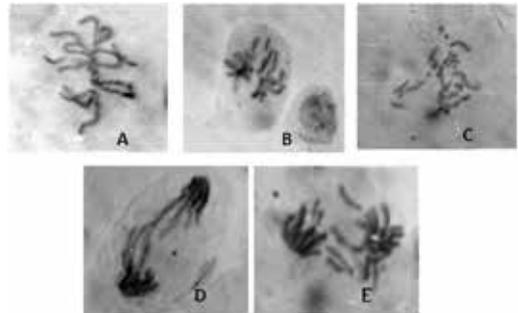


Mitotic study: The data obtained has been summarized in table (2) with detail study of the effect of plant extract on mitotic index. The extract of *Terminalia chebula* had a strong mitostatic effect on *Vicia faba* root as evident by the mitotic index which decreases with increase in duration 4- 24 hrs and concentration from 5 to 50 %.

Statistical analyses indicate that all values for 24hrs observations were significant at p value $P < 0.05$ in comparison to control.

Various types of abnormalities were observed viz., condensed and sticky chromosomes, disturbed anaphase, fragments, chromatin bridges, laggards. These observations may be due to nucleotoxic action of extracts or the disturbance of the formations of spindle fibers during cell division, which leads to chromosomal aberration (Figure 2).

Figure 2: Effect of high doses of Terminalia chebula extract on mitotic chromosome- A. Metaphase: Disturbed & Sticky chromosome B. Metaphase : Sticky chromosome arranged in two groups with fragments C. Anaphase : Disturbed arranged in three groups along-with fragments D. Late Anaphase: two broken bridges. E. Anaphase: Clumped with lagging chromosome.



DISCUSSION

It is suggested that mutagenic data from plant assays are very important for genetic research as well as for maintaining a stable ecosystem. In present study the chromosomes of *Vicia faba* observed with various abnormalities in chromosome after treated with extract of *Terminalia chebula*. The toxic effects can be observed at the level of chromosome structure (clastogenesis) through alterations in chromosome structure (chromosomal aberrations or CA) and number (aneuploidy, polyploidy). The clastogenic effects caused by the extracts from plant extracts species included anaphase/telophase bridges, chromosome fragments and sticky chromosome.

Meiosis of untreated control plant is normal showing six bivalents ($2n = 12$). Treated plants were showed sticky multivalents in the form of tri, quadri, hexa and octavalent (Figure 1: A, C) and that was more frequent at higher concentrations. In flower bud treatment, abnormalities ranged from 0.65% to 1.2%. Polysomic inheritance could cause due to multivalent formation. Individual phyto-chemical of *Terminalia chebula* extract must be studied for such toxicity.

Chromosomal stickiness was characterized by intense chromosome clustering during any phase of cell cycle as defined by Koernicke (1905). Babich et al., (1997) reported that metaphases with sticky chromosomes lose their normal appearance and appear to have a sticky "surface" which causes chromosome agglomeration, possibly due to effects on chromatin and chromosome organization. The stickiness of chromosomes at diakinesis (Figure 1: D) were observed. Frequency was high in metaphase I in *Vicia faba* (5.01%). The stickiness of chromosome could be induced by *T. chebula* by disrupt the bonds between protein of chromosomal surface and nucleic acids constituents of chromosomes (Babich et al., 1997). The distorted proteins become thick and develop sticky bridges by interfering the normal arrangement of chromosomes at metaphase. The chromosomes with bridges have inability to separate. However the spindle fibres pulled the chromosomes towards the poles these bridges were broken into fragments, which either moved towards the poles or formed laggards and micronuclei. During stickiness chromosomes formed a compact mass and the identity of individual chromosomes was lost. The manifestation of stickiness ranged from mild to intense followed by chromosome degeneration. Stickiness taken together with abnormal spindles, irregular chromosome segregation, pycnotic nuclei were considered the most probable causes of lower fertility.

The presence of chromosome fragments appeared due to breakage in chromosome, which can be a result of anaphase/ telophase bridges. The chromatin bridges with or without fragment (figure 1, B & D) was noticed in *Vicia faba*. The frequency of bridges was observed upto $2.05\% \pm 0.244$. Chromatin bridges were observed in high frequency at Anaphase I and low frequency at Anaphase II (figure 1: B, D). The bridge was formed due to sticky nature of chromosomes. The bivalent could not move at anaphase I to their respective poles due to inactivation of spindle and sticky nature got stretched up due to attracting forces of the poles (Nwankiti, 1970; Newell et al., 1984). Single or double bridges were known to result from asymmetrical length of chromatids or chromosomes inter changes (Dixit and Dubey, 1986).

Multipolar anaphases and telophases (Multipolar) are cells in anaphase and telophase stages that have more than two spindle poles instead of the normal two (Figure 1 B). The order of prevalence of the different types of aberrations that were induced is presented in Table 1. The order of frequency at higher dose (30%-50%) was: stickiness ($5.01\% \pm 0.618$) > multipolarity ($2.6\% \pm 0.403$) > anaphase and telophase bridges (2.05 ± 0.244) > Fragments ($1.55\% \pm 0.265$) > Multivalents ($1.21\% \pm 0.198$) > chromosome laggards ($0.95\% \pm 0.14$). The results were in accordance to effect of pesticides reported by (Asita and Mokhobo, 2013) and heavy metals reported by (Tripathi & Kumar, 2010). These anomalies revealed that the extract concentration effects on both chromosome structure and spindle material (Khandelwal, 1986). Because abnormalities of the cell division process results from the genotoxic effects, the phytochemicals from *T chebula* have the potential at higher dose to cause adverse effect on human health. Pollen grains produced by cells with meiotic stickiness are generally non-viable for fertilization because they are unbalanced through irregular chromosome segregation and fragmentation as found in pearl millet (Rao et al. 1990).

Our study reports that *T chebula* is non-toxic at lower doses while higher doses may be clastogenic but still the toxicity of pesticides and heavy metals is more considerable.

The reduction in mitotic index (8.602 ± 0.019 to 7.100 ± 0.015) might be due to turbagenic changes induced by the extract in nuclear chromatin (Asthana et al., 2011). The reduction of the mitotic index can be explained by the arrest of the division of the interphasic nucleus, as well as by death of interphasic nucleus, hindering the onset of the prophase and, thus, the division of the cells (Sousa et al., 2010). Mitotic index is considered a parameter that allows one to estimate the frequency of cellular division (Marcano et al., 2004) and the reduction of mitotic activities has been used frequently to trace substances that are cytotoxic (Linnainmaa et al., 1978; Smaka-Kincl et al., 1996). Previously *T chebula* was not found genotoxic in VITOTOX Test and Ames test while COMET assays shown it as genotoxic at content more than 500 ppm (Arora et al 2005). These assays are useful to detect DNA damage and mutation in prokaryotic and eukaryotic cells.

The total of abnormalities recorded at different treatment, increases ($9.87\% \pm 0.025$ to $20.40\% \pm 0.029$) as the concentration increase (5% to 50%) and time prolonged. The total abnormalities was increase up to 12hrs then recovered by cells. However, in all time period frequency of abnormalities increased significantly at $P < 0.05$ with increase in concentration of extract when compared to control cells. Strongly we could conclude that there is strong negative relationship between MI and the percentage of abnormalities as previously recorded for many extracts (Singh et al 1989; Sousa et al 2010).

Stickiness and clumping of chromosomes were the most frequent effect of extract (Figure 2, A & B). Stickiness usually leads to formations of anaphase, metaphase and telophase bridges. Stickiness might be due to DNA depolymerisation and partial dissolution of nucleoprotein proteins and the stripping of the protein covering of DNA in chromosomes as also observed by Onyenwe, (1983).

Numerical and structural changes in chromosomes were attributed to spindle failure, leading to fragmentation, lagging chromosomes (Singh, 1982). Fragments were noticed either due to terminal breaks in the chromosome or failure of chromosome thread to rejoin (Figure 2: B & C). Bridge was observed due to sticky nature of chromosomes (Fawzia et al., 2012) (Figure 2: D). The lagging chromosome or laggards (Figure 2: E) was possibly formed due to the inhibition of centromeric or spindle activity which inhibits chromosome movements and due to presence of acentric fragment or the interaction of extract with protein of spindle apparatus (Wuu and Grant, 1967).

These aspects were observed mainly with the increment of the concentrations of the extracts, where the mitotic index showed a significantly decrease in relation to the control. Similar results were observed in previous studies about other medicinal plant extracts and the cell death was considered the major depressor of the mitotic index (Çelik and Aslantürk, 2006 & 2007; Campos et al. 2008; Lubini et al. 2008).

Root tip treatment indicates significant abnormalities at $P < 0.05$ (Table 1 & 2) that was subjected to varying concentration of extract. The MI values were differed significantly at $P < 0.05$ than control. Types of abnormalities were same both mitotic and meiotic cells; in all cases sticky, multi-polar and bridge type anomalies were frequent. The possible mechanism for the induction of above aberrations is directly or indirectly concerned with the action of higher doses of *Terminalia chebula* extract on DNA damage.

The present study, strongly recommend that one should calculate a suitable dose to folk medicines before administration. Because modifications of cell division process often results in the production of daughter cells with abnormal chromosome numbers, thus administration of injudicious higher doses of *Terminalia chebula* could be a potential cause of adverse effects.

It is concluded that inadvisable higher concentration of *Terminalia chebula* induces cytotoxicity in DNA of *Vicia faba*. Thus, results of the present study indicate that use of medicinal plants without any consultation of physician may become harmful for health.

ACKNOWLEDGEMENT

Authors are thankful to Prof. Rajendra Sharma, Head, Dept of Botany, School of Life Sciences, Khandari Campus, Dr. Bhim Rao Ambedkar University, Agra, for his constant support during the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Table 1: MEIOTIC CHROMOSOMAL ABNORMALITIES INDUCED BY DIFFERENT CONCENTRATION OF TERMINALIA CHEBULA (FLOWER BUD TREATMENT) IN VICIA FABEA

	Conc %	Sticky chro.	MPS	Multivalents	Frag.	C.B.	La.	Total
24hrs	Control	0.127	0.000	0.000	0.000	0.000	0.000	0.127
	5	2.199	0.000	0.000	0.000	0.542	0.000	2.741
	10	2.663	0.000	0.000	0.000	0.746	0.000	3.408
	20	2.826	0.720	0.000	0.000	0.858	0.329	4.733
	30	2.984	0.928	0.431	0.696	0.862	0.000	5.901
	40	3.007	1.298	0.601	0.791	1.044	0.000	6.741
	50	3.021	1.568	0.724	0.935	1.236	0.573	8.056

Note : Conc % = Concentration in percentage; Sticky chro = sticky chromosome; MPS = Multipolar spindles; Frag = Fragments; CB = Chromatin bridges; La = Laggards; Total = Total of abnormalities. All values for 24hrs observations were found significant at p value $P \leq 0.05$ in comparison to control.

Table 2: TYPE AND DISTRIBUTION OF CHROMOSOMAL ABNORMALITIES INDUCED BY DIFFERENT CONCENTRATION AND DURATION OF TERMINALIA CHEBULA (ROOT-TIP TREATMENT) IN VICIA FABA –

Du.	Conc. %	MI	Cond SC	MPS	Frag	CB	La	Total
4hrs	Control	8.724	1.810	0.000	0.000	0.000	0.000	1.810
	5	8.610	6.417	0.457	0.411	0.519	0.000	7.804
	10	8.519	6.940	0.000	0.747	0.622	0.000	8.309
	20	8.461	7.979	0.000	0.803	0.711	0.000	9.494
	30	8.775	8.463	0.000	1.037	0.780	0.000	10.280
	40	8.280	10.525	0.000	1.065	1.012	0.000	12.602
	50	8.602	11.382	0.000	1.290	1.329	0.522	14.001
8hrs	Control	10.851	1.956	0.000	0.000	0.000	0.000	1.956
	5	7.983	7.290	0.000	0.467	0.532	0.000	8.289
	10	8.761	9.511	0.000	0.725	0.660	0.000	10.897
	20	8.503	9.708	0.000	0.809	0.833	0.000	11.350
	30	8.240	9.800	0.000	0.920	1.104	0.000	11.824
	40	7.729	9.402	1.188	0.931	1.143	0.000	12.665
	50	7.613	9.503	0.375	1.613	1.538	1.418	13.028
12hrs	Control	9.696	1.200	0.000	0.000	0.000	0.000	1.200
	5	8.516	6.518	0.000	0.806	0.474	0.000	7.797
	10	8.268	8.923	0.000	0.967	0.764	0.000	10.655
	20	8.008	10.173	0.000	1.051	1.353	0.000	12.577
	30	7.926	10.327	1.320	1.121	1.455	0.000	14.223
	40	7.503	10.296	0.986	1.207	1.578	0.000	14.068
	50	7.273	10.386	0.394	1.277	1.615	0.827	13.672
24hrs	Control	8.130	1.500	0.000	0.000	0.000	0.000	1.500
	5	8.178	6.118	0.000	0.000	0.426	0.000	6.544
	10	8.025	6.862	0.000	0.000	0.658	0.000	7.520
	20	7.496	7.126	0.000	0.733	0.733	0.000	8.591
	30	7.702	7.998	0.000	0.801	1.027	0.000	9.826
	40	7.556	8.796	0.000	0.000	1.170	0.465	9.967
	50	7.100	9.513	0.472	0.000	1.408	0.000	11.392

Note: Du. = Duration; Con % = Concentration in percentage; MI = Mitotic index (Number of dividing cell/1000 scored); MI= Mitotic Index; Cond SC = Condensed and sticky chromosomes; MPS = Multipolar spindles; Frag = Fragments; CB = Chromatin bridges; La = Laggards; Total = Total of abnormalities.

REFERENCE

- [1] Arora, S., Brits, E., Kaur, S., Kaur, K., Sohi, R.S., Kumar, S., Verschaeve, L. (2005). Evaluation of genotoxicity of medicinal plant extracts by the Comet and VITOTOX tests. *J Environ Pathol Toxicol Oncol* 24(3):193-200. | [2] Asita, A.O., Mokhobo, M.M. (2013). Clastogenic and Cytotoxic Effects of Four Pesticides Used to Control Insect Pests of Stored Products on Root Meristems of *Allium cepa*. *Environ. Nat. Resour. Res* 3(2):133-145. | [3] Asthana, M., Kumar, A., Sharma, S. (2011). Cytogenetic effects of *Terminalia bellerica*, Roxb. on root meristem of *Vicia faba*. *Adv. Biores.* 2(1):21-25. | [4] Asthana, M., Kumar, A. (2014). Dose Response of *Viola odorata* on Meiotic and Mitotic Chromosomes of *Vicia faba*. *British J Pharm. Res.* 4(4): 520-530. | [5] Babich, H., Segall, M.A., Fox, K.D. (1997). The *Allium* test—a simple, eukaryote genotoxicity assay. *Am. Biol. Teach.* 59(9): 580-583. | [6] Bag, A., Bhattacharyya, S.K., Chattopadhyay, R.R. (2013). The development of *Terminalia chebula* Retz. (Combretaceae) in clinical research. *Asian Pac. J. Trop. Biomed.* 3(3):244-252. | [7] CaMpos, J.M.S., DaviDe, L.C., Soares, G.L.G., Viccini, L.F. (2008). Mutagenic effects due allelopathic action of fern (*Gleicheniaceae*) extracts. *Allelopathy J.* 22:143-152. | [8] Çelik, T.A., Åslanturk, Ö.S. (2006). Anti-mitotic and anti-genotoxic effects of *Plantago lanceolata* aqueous extracts on *Allium cepa* root tip meristem cells. *Biologia.* 61:693-697. | [9] Çelik, T.A., Åslanturk, Ö.S. (2007). Cytotoxic and genotoxic effects of *Lavandula stoechas* aqueous extracts. *Biologia.* 62:292-296. | [10] Darlington, C.D., LaCour, L.E. (1976). The handling of chromosomes. Sixth edition. George Allan and Unwin Ltd., London. | [11] Dixit, P., Dubey, D.K. (1986). Mutagenic efficiency of gamma rays, NMU and their combination in lentil (*Lens culinaris* Med.) *Var. T. 36. Ind. J. Genet.* 43(3):501-505. | [12] Fawzia, I., Mohamed, El-Ashry, Z.M. (2012). Cytogenetic effect of allelochemicals *Brassica nigra* L. extracts on *Pisum sativum* L. *World Applied Sci. J.* 20(3):344-353. | [13] Gulfishan, M., Khan, A.H., Bhat, T.A. (2010). Studies on cytotoxicity induced by DES and SA in *Vicia faba* *Var. major*. *Turk. J. Bot.* 34:31-37. | [14] Haroun, S.A., Al Shehri, A.M. (2001). Cytogenetic effects of *Calotropis procera* extracts on *Vicia faba* L. *Cytologia* 66:373-378. | [15] Inamdar, M.C., Rao, R.M.R. (1962). Studies on the pharmacology of *Terminalia chebula*[J]. *J. Sci. Ind. Res.* 21C:345-348. | [16] Khandelwal, S. (1986). Effect of Ophioglossum Oil on mitosis in *Allium sativum* and *Vicia faba*. *Cytologia* 51:579-586. | [17] Koehn, F.E., Carter, G.T. (2005). The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.* 4:206-220. | [18] Koernicke, M. (1905). Über die Wirkung von Rotgen und Radiumstrahlen auf Pflanzliche Gewebe und Zellen *Dtsch Bot. Ges.* 23:404-415. | [19] Lee, H.S., Koo, Y.C., Suh, H.J., Kim, K.Y. Lee, K.W. (2010). Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation end product-induced endothelial cell dysfunction. *J. Ethnopharmacol.* 131(3): 567-74. | [20] Linnainmaa, K., Meretoja, T., Sorsa, M., Vainio, H. (1978). Cytogenetic effects of styrene and styrene oxide. *Mutation Res.* 58(2-3):277-286. | [21] Lubini, G., Fachinnetto, J.M., Laughinghouse, H.D., Paranhos, J.T., Silva, A.C.F., Tedesco, S.B. (2008). Extracts affecting mitotic division in root-tip meristematic cells. *Biologia.* 63:647-651. | [22] Marcano, L., Carruyo, I., Del campo, A., Montiel, X. (2004). Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L. *Environ. Res.* 94(2):221-226. | [23] Newell, C.A., Rhoads, M.L., Bidney, D.L. (1984). Cytogenetic analysis of plants regenerated from tissue explants and mesophyll protoplasts of winter rape, *Brassica napus* L. *Can. J. Gen. Cytol.* 26:752-761, 10.1139/g84-119. | [24] Nwankiti, O. (1970). Cytogenetics and breeding studies with *Brassica* I. Cytogenetic experiments with *Brassica napocampestris*. *Hereditas* 66:109-126. | [25] Onyenwe, C.N. (1983). Cytological effects of seed extracts of *Abrus procatorius* on the mitosis of *Allium cepa* and the effect of root extract of *Boerhaavia diffusa* on mitosis of *Crassocephalum bialfræ*. University of Port Harcourt, Port Harcourt. | [26] Rangari, V.D. (2007). Tannin Containing Drugs. *Pharmacognosy. NISCAIR Govt of India, New Delhi.* (<http://nsdl.niscair.res.in/bitstream/123456789/591/1/revised+Tannins+containing+Drugs.pdf>) read on 31 aug 2013. | [27] Rao, P.N., Kanganadhaam, P., Nirmala, A. (1990). Behaviour of sticky desynaptic mutant in pearl millet. *Genetica* 81:221-227. | [28] Roy, P., Amdekar, S., Kumar, A., Singh, R., Sharma, P., Singh, V. (2012). In vivo antioxidative property, antimicrobial and wound healing activity of flower extracts of *Pyrostegia venusta* (Ker Gawl) Miers. *J. Ethnopharmacol.* 140(1):186-92. | [29] Singh, M.R. (1982). Effects of IAA with maleic hydrazid and colchicine on root tip mitosis. *Cytologia.* 47:419-426. | [30] Singh, R.M., Sarbhoy, R.K., Sharma, A. (1989). Mitotic instability induced by organophosphorous pesticides in onion (*Allium cepa*). *Proceedings of 76th Indian Science Congress*, pp-198. | [31] Smaka-Kincl, V., Stegnar, P., Lovka, M., Toman, M.J. (1996). The evaluation of waste, surface and ground water quality using the *Allium* test procedure. *Mut. Res.* 368:171-179. | [32] Sousa, S.M., Silva, P.S., Viccini, L.F. (2010). Cytogenotoxicity of *Cymbopogon citratus* (DC) Stapf (lemon grass) aqueous extracts in vegetal test systems. *An Acad. Bras. Ciênc.* 82(2):305-311. | [33] Surh, Y., Ferguson, L.R. (2003). Dietary and medicinal anti-mutagens and anti-carcinogens: Molecular mechanisms and chemo-preventive potential - highlights of a symposium. *Mut. Res.* 523-524:1-8. | [34] Surya Prakash, D.V., Sree Satya, N., Avaniagadda, S., Vangalapati, M. (2012). Pharmacological Review on *Terminalia Chebula*. *Int. J. Pharm. Biomed. Res.* 3(2): 679-683. | [35] Tripathi, R., Kumar, G. (2010). Genetic loss through heavy metal induced chromosomal stickiness in Grass pea. *Caryologia* 63(3):223-228. | [36] Vani, T., Rajani, M., Sarkar, S., Shishoo, C.J. (1997). Antioxidant properties of ayurvedic formulation triphala and its constituents. *Int. J. Pharmacog.* 35:313-317. | [37] Wu, K.D., Grant, W.F. (1967). Chromosomal aberrations induced in somatic cells of *Vicia faba* by pesticides. *Nucleus* 10(1):37-46. |