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GENOTOXIC ASSESSMENT OF SEED EXTRACTS OF *THEVETIA PERUVIANA* PERS. AND *CERBERA ODOLLAM* GAERTN. USING CHROMOSOME ABERRATION STUDY

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The use of plants by the general population is an old and still wide spread practice which makes studies of their genotoxicity essential. The effects of aqueous seed extracts of *Thevetia peruviana* Pers. and *Cerbera odollam* Gaertn was evaluated using *Allium cepa* chromosome aberration study. Seed extracts suppressed the mitotic activity of *Allium* root meristems after 1, 3 and 5 hours treatment with all concentrations. *Thevetia* extract was the most effective in its ability to inhibit cell division compared with *Cerbera*. Analysis of data of phase index indicates that all treatments reduced prophase percentage, meanwhile the percentage of meta, ana and telophase increased than those of control. Also the extracts caused different kinds of chromosome aberrations in dividing and non dividing cells such as chromosome bridges, stickiness, mis congression, laggards and other disturbed anomalies. Bridges was the most common aberration in dividing cells. The infusions at higher concentrations caused a statistically significant inhibition of cellular division on the onion cells. Recovery studies after 24 hours in water confirm that the cerebral glycosides present in the extracts was cytotoxic rather than cytostatic. These results regarding the cytotoxicity and mutagenicity of these plants provide valuable information about the safety of using them as therapeutic agents.

Keywords: *Cerbera odollam* Gaertn; Chromosome aberration; Cytotoxic; Genotoxic; Mitotic index; *Thevetia peruviana* Pers.

Introduction

Higher plants as source of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50% of all modern clinical drugs are of natural product origin and they play an important role in drug programmes of the pharmaceutical industries[1]. However, medicinal plants, and indeed plants in general, synthesize toxic substances, which in nature act as a defense against infections, insects, and herbivores, but which often affect the organisms that feed on them. Thus, an assessment of their cytotoxic and mutagenic potential is necessary to ensure a relatively safe use of medicinal plants. Researchers studied the mode of action, toxicity, physiology and cytology of many plant extracts such as colchicine, caffeine, mimosin, morindine and many others[2,3].

The species *Thevetia peruviana* Pers. and *Cerbera odollam* Gaertn. are examples of poisonous plants often used in popular medicine belongs to the family Apocynaceae. The yellow oleander's leaves, flowers, milky latex and especially the seeds are extremely poisonous. The plant contains various cardiac glycosides of which thevetin A and B have previously been mixed as a commercial heart stimulant. The symptoms of ingesting any part of the yellow oleander include vomiting, diarrhoea, stomach pain and heart related problems. Death may occur rapidly if large quantities of the plant are ingested, however, the bitter taste is a deterrent and vomiting usually prevents serious damage. In South Africa, yellow oleander is listed as an invader due to its competitive nature and toxicity. It occurs naturally in Mexico, tropical America and the West Indies and was brought to South Africa as an ornamental shrub. It has now invaded savannah, watercourses and coastal regions, mainly in the sub-tropical regions. *Cerbera* contain cerberin another cerebral glycoside used as a suicide poison and to stupefy fish by the Philippines.

Materials and Methods

Seed samples of *Thevetia peruviana* Pers. and *Cerbera odollam* Gaertn. were obtained from plants growing wild along the Velli lake. The infusions were prepared by crushing the seed and extract them with water. 100 g powder stepped over night in a cloth bag suspended in distilled water. The resulting crude suspension was used for the preparation of different concentrations of seed extract. The concentrations are 0.5, 1, 1.5, 2 and 2.5 % (containing 5000, 10000, 15000, 20000 and 25000 ppm respectively). The *Allium* test was carried out as described by Rank and Nielsen[4]. Commercial onion bulbs of *Allium cepa*, were obtained from National institute of Agriculture, New Delhi.

Before use, the loose outer scales were carefully removed, and the dry bottom plates were scraped without destroying the root primordial. For each sample 12 onions were set up and allowed to produce roots in tap water for 2 days. On the second day they are transferred to the test solutions. Roots (2-3 cm) were treated with test solutions of 0.5, 1, 1.5, 2 and 2.5 % for 1, 3 and 5 hours, while the control group was left in tape water for the same period of time. After treatment, six onion bulbs were fixed immediately in acetoalcohol (1:3). The other six bulbs were returned to water, for a further 24 hour, to observe if there was recovery from possible damage. After fixation, the slides were prepared for examination using 5-6 root tips as per the methodology of Kao[5] examined and photographed. The mitotic index (MI), phase index and chromosome aberrations were determined by examining 1000 cells at least. The data were statistically analyzed by the conditional test for the detection of rare events, with the level of significance set at 0.05.

Results and Discussion

The use of biological active substances from yellow oleander and *Cerbera* as therapeutic agents has been undertaken in different parts of the world. Table 1 shows the results obtained with the seed infusion of *Thevetia*, the percentage total mean mitotic index (MI), the total number of analyzed cells, and the number of cells in each one of the different phases of cell cycle (interphase, prophase, metaphase and telophase) for each group of the six onions, the controls and the treated plants. Table 2 shows the results obtained with *Cerbera* seed extract for the three concentrations. In this study, seed extracts suppressed the mitotic activity of *Allium* root meristems after 1, 3 and 5 h treatment with all concentrations. Njagi and Gopalan[6] reported that the effect of food preservatives, sodium sulphate and sodium benzoate might be related to inhibitory effect on DNA synthesis. Similarly the effect of seed extracts might interact with DNA nucleotide thus inhibiting DNA synthesis (DNA / nucleoprotein equilibrium) and subsequent mitotic inhibition. In general *Cerbera* seed extract was less effective in its ability to inhibit cell division compared with *Thevetia*. However, this antimitotic effect was too drastic in both the cases as cell growth was unable to recover after 24 hour in water. Thus the infusions in the *Allium* root meristem test system was cytotoxic rather than cytostatic, since there was no recovery of cell division after replacement of the plant infusion by water for 24 hours.

Researchers showed that the mitotic arrest could be due to change in the duration of the mitotic cycle such as increase in the G_2 period[7,8] or increase in S phase duration[9]. This occurred probably through the uncoupling of respiratory processes and carbohydrate metabolism leading to low ATP content, which is essential for mitotic process[10]. The inhibition of cell division by natural compounds present in the plant extract has been reported by Adam[9], and Cherian[10]. Studying the data of phase index it is obvious that all the treatments reduced the percentage of prophase mean while the meta, ana and telophase percentage rose over those of control suggesting clearly that the chemicals of the extract may interfere with or lead to breakage of DNA synthesis rather than the retardation of the spindle formations, where MI depression was not accompanied by lowering the meta, ana and telophase percentage. Treatments significantly increased the percentages of aberrant in dividing and non dividing cells of *Allium* meristems than their respective control.

Nine major types of chromosomal aberrations were recorded; these are mitotic arrest, pycnotic nuclei, stickiness and clumping, sticky bridges, achromatic lesions, mis congression of chromosomes, ball metaphase, clastogenic effects and spindle abnormalities (Table 3 and 4). The most common aberration is clastogenic effects manifested as chromosome bridges and fragments. Bridges one, two or multiple were recorded in ana and telophase configuration (Fig. 1-9). They are the results of breaks with subsequent reunion of chromosomes / dicentric chromosomes or stickiness of chromosomes. The sticky bridges resulted may be by the depolymerization and cross linking of DNA of the chromosomes and consequent failure of anaphase separation[11,12]. During the present study few cells showed fragments and lagging chromosomes. Condiments have been found to induce fragmentation[13]. Compounds inhibit nucleic acid synthesis leads to produce varying degree of chromosomal fragmentation[14-16]. The dark and light stained regions in prophase chromosomes – pycnotic nuclei were observed in a few cells. Similar observations in garlic root cells treated with cigar constituents were reported by Arnold *et al*[17] (Fig.10).

Chromosome stickiness and clumping was also another type of abnormalities observed in meta and anaphase cells. This may be due to heterochromatinisation by depolymerization or partial dissolution of nucleoprotein of the chromosomes, causing the denaturation of DNA making the surface hazy and adhesive or disturbance in nucleic acid or protein metabolism by disturbed RNA synthesis resulting in the alternation of chromosomal nucleoprotein configuration or improper folding of chromosome fiber[17] (Fig. 11-15). Presence of achromatic lesions in the form of unstained regions at inter and prophase in the treated cells may due to lost chromosomal materials / alternations in chromosomal packing / the points where union of broken ends occurred / due to localized uncoiling of chromosomes[18] (Fig. 16-20).

Moreover, cells containing mis congression

Table 1. Mitotic index (MI), phase index in each phase and interphase cells in treated *Allium cepa* root with different concentrations and different period treatment with yellow oleander seed extracts.

	Control	0.50%	1%	1.50%	2%	2.50%
			1 Hour Treatment			
Total divided % ± SE	114.4 ± 3.9*	80.5 ± 7.0*	70.1± 3.2*	60.6 ± 6.7*	55.8 ± 3.4*	39.1 ± 5.1*
MI % ± SE	11.4 ± 0.4*	8.1 ± 0.7*	7 ± 0.32*	6.0 ± 0.67*	5.6 ± 0.34*	3.91 ± 0.51*
Pro.P % ± SE	29.0 ± 4.2	20.7 ± 4.0NS	25± 5.6NS	25.9 ± 4.7NS	29.6 ± 6.4NS	37.2 ± 18.5NS
Meta.P % ± SE	24.0± 3.8	33.7 ± 5.1NS	35± 7.4NS	43.5 ± 6.3NS	40.6 ± 7.2NS	13.2 ± 11.3NS
Ana.P % ± SE	24.0 ± 5.2	27.1 ± 4.2*	23.3 ± 6.2NS	12.9 ± 3.2NS	15.8 ± 4.6NS	25.2 ± 1 5.1NS
Telo.P % ± SE	33.9 ± 5.9	19.3 ± 3.8NS	16.7± 5.2NS*	17.6 ± 5.3NS	17.8 ± 7.4NS	23.2 ± 19.7NS
			3 Hours Treatment			
Total divided % ± SE	109.2 ± 4.9*	79.5 ± 7.2*	65.5 ± 4.5*	83.6 ± 7.2*	49.6 ± 4.1*	43.2 ± 3.1
MI % ± SE	10.9 ± 0.5*	8 ± 0.7*	6.6± 0.45*	8.4 ± 0.7*	4.96 ± 0.41*	4.32 ± 0.31
Pro.P % ± SE	25.6 ± 3.6	18.2 ± 5.8NS	17.1 ± 5.8 NS	15.5 ± 3.8*	17.1 ± 2.6*	18.2 ± 4.2*
Meta.P % ± SE	22.3 ± 4.3	28.5 ± 6.6NS	30.5 ± 5.1*	27.9± 4.9*	30.1 ± 5.4*	31.9 ± 6.5NS
Ana.P % ± SE	28.6 ± 7.0	25.6 ± 6.3NS	23.2 ± 4.9NS	20.3 ± 6.6NS	20.4 ± 4.9NS	26.1 ± 5.6NS
Telo.P % ± SE	30.7 ± 6.4	23.1 ± 4.4*	19.7 ± 2.9*	13.3 ± 5.2*	16.6 ± 3.8*	12.2 ± 5.4NS
			5 Hours Treatment			
Total divided % ± SE	110 ± 4.9*	58.4 ± 3.9*	60.2 ± 3.5*	48.1 ± 7.0*	35.6 ± 4.5*	20.6 ± 4.9
MI % ± SE	11.0 ± 0.5*	5.8 ± 0.39*	6.0± 0.35*	4.8 ± 0.7*	3.56 ± 0.45*	2.06 ± 0.49
Pro.P % ± SE	23.2 ± 2.6	24.8 ± 4.3NS	17.5 ± 4.0 NS	26.8 ± 4.1NS	23.3 ± 3.4*	31.6 ± 9.2NS
Meta.P % ± SE	24.6 ± 4.3	29.3 ± 5.0NS	23.8 ± 5.0NS	29.3 ± 5.3NS	52.3 ± 9.9NS	51.6 ± 11.2NS
Ana.P % ± SE	26.5 ± 5.0	17.0 ± 5.3NS	22.5 ± 4.4NS	17.3 ± 5.3NS	13.8 ± 4.6NS	10.1± 14.3NS
Telo.P % ± SE	28.7 ± 5.4	24.7 ± 4.9NS	28.8 ± 6.1NS	56.4 ± 11.8NS	5.3 ± 1.7*	4.1± 16.9NS

Table 2. Mitotic index (MI), phase index in each phase and interphase cells in treated *Allium cepa* root with different concentrations and different period treatment with *Cerbera odallam* seed extracts.

			1 Hour Treatment			
Total divided % ± SE	114.4 ± 3.9*	91.5 ± 8.0*	56.1± 5.2*	80.4 ± 6.7*	57.8 ± 4.1*	39.2 ± 5.1*
MI % ± SE	11.4 ± 0.4*	9.1 ± 0.8*	5.6± 0.52*	8.0 ± 0.67*	5.8 ± 0.41*	3.92 ± 0.5a1*
Pro.P % ± SE	29.0 ± 4.2	7.1 ± 0.5*	16.1± 5.2*	13.6 ± 6.0*	17.8 ± 3.4*	13.2 ± 3.5*
Meta.P % ± SE	24.0 ± 3.8	29.5 ± 4.6*	26.1± 3.4*	23.6 ± 4.7*	27.8 ± 1.2*	33.2 ± 3.3*
Ana.P % ± SE	24.0 ± 5.2	25.6 ± 6.8*	25.3 ± 6.2*	22.5 ± 5.2*	37.8 ± 5.6*	33.2 ± 5.1*
Telo.P % ± SE	33.9 ± 5.9	39.1 ±3.0*	36.4± 2.9*	33.6 ± 7*	37.8 ± 3.4*	43.2 ± 9.7*
			3 Hour Treatment			
Total divided % ± SE	109.2 ± 4.9*	70.9 ± 5.0*	65.5 ± 4.5*	83.6 ± 7.2*	49.6 ± 4.1*	43.2 ± 3.1
MI % ± SE	10.9 ± 0.5*	8 ± 0.7*	6.6± 0.45*	8.4 ± 0.7*	4.96 ± 0.41*	4.32 ± 0.31
Pro.P % ± SE	25.6 ± 3.6	12.2 ± 2.8*	15.6 ± 5.8 NS	11.0 ± 3.8*	17.1 ± 2.6*	18.2 ± 4.2*
Meta.P % ± SE	22.3 ± 4.3	29.5 ± 4.6*	41.2 ± 5.1*	47.9± 6.9*	37.1 ± 5.5*	34.9 ± 7.5NS
Ana.P % ± SE	28.6 ± 7.0	25.6 ± 6.8*	34.4 ± 4.8*	21.9 ± 4.6NS	25.7 ± 4.9NS	27.5 ± 5.6NS
Telo.P % ± SE	30.7 ± 6.4	39.1 ± 3.0*	10.0 ± 2.9*	19.2 ± 3.2*	20.0 ± 4.8*	19.3 ± 5.7NS
			5 Hour Treatment			
Total divided % ± SE	110 ± 4.9*	68.4 ± 5.2*	60.2 ± 3.5*	78.7 ± 7.0*	45.5 ± 5.5*	39.5 ± 3.1
MI % ± SE	11.0 ± 0.5*	6.8 ± 0.5*	6.0± 0.35*	7.8 ± 0.7*	4.55 ± 0.55*	4.0 ± 0.31
Pro.P % ± SE	23.2 ± 2.6	14.4 ± 3.3*	17.5 ± 4.0 NS	6.8 ± 2.1*	9.9 ± 3.4*	21.2 ± 5.2*
Meta.P % ± SE	24.6 ± 4.3	28.9 ± 5.4*	23.8 ± 5.0NS	56.8± 7.3*	23.9 ± 6.4*	47.4 ± 6.9NS
Ana.P % ± SE	26.5 ± 5.0	32.0 ± 4.8*	22.5 ± 4.4NS	0	33.8 ± 5.6*	0
Telo.P % ± SE	28.7 ± 5.4	24.7 ± 4.9NS	38.8 ± 5.1NS	36.4 ± 6.8NS	31 ± 5.7NS	31.6 ± 6.1NS

Table 3. Percentage of abnormalilties in each phase and interphase cells in treated *Allium cepa* root with different concentrations and different period treatment with yellow oleander seed extracts.

		1 Hour Treatment (in percentage)				
		0.50	1.00	1.50	2.00	2.50
% of abnc		11.2	11.8	21.7	25	28
Inter.p	Multi.N	0	0.3	0.2	2	0
Pro.p	Micro.N	0	13.3	0	68.4	66.7
Meta.p	Non Cong.	0	4.8	0	11.5	0
	Micro.N	0	0	0	7.7	0
Ana.p	Micro.N	0	0	0	40	0
	Bridge	32	0	18.2	10	0
	Disturbed	12	21.4	27.3	0	0
	Chrom.Rin	3.2	0	0	0	0
	Stickiness	16.1	0	0	0	0
Telo.p	Laggard	0	0	0	9.1	0
	Micro.N	0	0	0	45.5	0
	Bridge	0	0	0	9.2	0
	Disturbed	5.9	10	33.3	0	0
	Diagnol	8	8	0	0	0
		3 Hours Treatment (in percentage)				
% of abnc		17.2	21.5	29.5	34	36
Inter.p	Multi.N	0.1	0.16	0.2	1.2	1.29
Pro.p	Micro.N	0	10	12.6	32	0
Meta.p	Non Cong.	0	0	0	0	0
	Micro.N	3	0	0	0	0
Ana.p	Micro.N	0	0	0	45.9	0
	Bridge	33	10	22	17.7	28.1
	Disturbed	4	0	0	29	0
	Chrom.Rin	0	0	0	0	0
	Stickiness	20	17.8	18	7.9	0
Telo.p	Laggard	3	0	4.4	10.4	0
	Micro.N	0	0	0	0	0
	Bridge	0	0	3	10.1	0
	Disturbed	6	10.9	29	12	0
	Diagnol	0	0	0	0	0
		5 Hours Treatment (in percentage)				
% of abnc		21.1	24	27	36	39.4
Inter.p	Multi.N	0	0.2	0.2	1.3	1.33
Pro.p	Micro.N	0	0	13	22	9
Meta.p	Non Cong.	4.1	1	2	5.3	5
	Micro.N	0	0	0	0	0
Ana.p	Micro.N	0	0	0	0	0
	Bridge	12	8.2	14.4	0	0
	Disturbed	0	0	1.1	0	4
	Chrom.Rin	0	0	0	0	0
	Stickiness	2.2	3.5	0	18	0
Telo.p	Laggard	0	3	0	6	0
	Micro.N	0	0	0	0	0
	Bridge	0	0	0	11	18.2
	Disturbed	0	0	0	0	0
	Diagnol	0	0	0	0	0

Table 4. Percentage of abnormalities in each phase and interphase cells in treated *Allium cepa* root with different concentrations and different period treatment with *Cerbera odallam* seed extracts.

		1 Hour Treatment (in percentage)				
		0.50	1.00	1.50	2.00	2.50
% of abnc		11.9	13	23	24.2	29.4
Inter.p	Multi.N	0	1	1.9	3.2	0.8
Pro.p	Micro.N	0	0	0	0	0
Meta.p	Non Cong.	0	0	6.8	0	13.1
	Micro.N	0	0	0	11	0
Ana.p	Micro.N	0	0	0	3.9	0
	Bridge	41	23.5	21	18	10
	Disturbed	0	0	0	0	0
	Chrom.Rin	0	0	0	0	0
	Stickiness	12	3.9	20	0	17.2
Telo.p	Laggard	1	0	1.8	4	3.1
	Micro.N	0.9	0	0	1	0
	Bridge	12	14.7	0	29	23.2
	Disturbed	3.4	0	0	0	0
	Diagnol	0	0	0	0	0
		3 Hours Treatment (in percentage)				
% of abnc		47.9	63.5	77.5	50	81.1
Inter.p	Multi.N	0	6	1.4	2.2	6.8
Pro.p	Micro.N	0	6	1.4	32	0
Meta.p	Non Cong.	0	0	8.3	9.1	0
	Micro.N	3	0	0	0	0
Ana.p	Micro.N	0	0	0	0	0
	Bridge	69	77.8	80	0	92
	Disturbed	0	0	0	6	0
	Chrom.Rin	0	0	0	0	0
	Stickiness	0	0	0	0	0
Telo.p	Laggard	2	0	1.5	4	0
	Micro.N	0	0	0	0	0
	Bridge	65.9	25	90	80	94.7
	Disturbed	0	0	0	0	0
	Diagnol	0	0	0	0	0
		5 Hours Treatment (in percentage)				
% of abnc		58.8	51.3	77.3	52.1	98.7
Inter.p	Multi.N	0.7	1.2	0.9	0.5	1.2
Pro.p	Micro.N	0	2	0	0	0
Meta.p	Non Cong.	17.9	0	24	0	0
	Micro.N	0	0	0	0	0
Ana.p	Micro.N	0	0	0	0	0
	Bridge	80.6	72.2	0	66.7	0
	Disturbed	0	0	0	0	0
	Chrom.Rin	0	0	0	0	0
	Stickiness	0	0	0	0	0
Telo.p	Laggard	0	0	0	0	0
	Micro.N	0	0	0	0	0
	Bridge	95.8	38.7	56.3	63.6	97.9
	Disturbed	0	0	0	0	0
	Diagnol	0	0	0	0	0

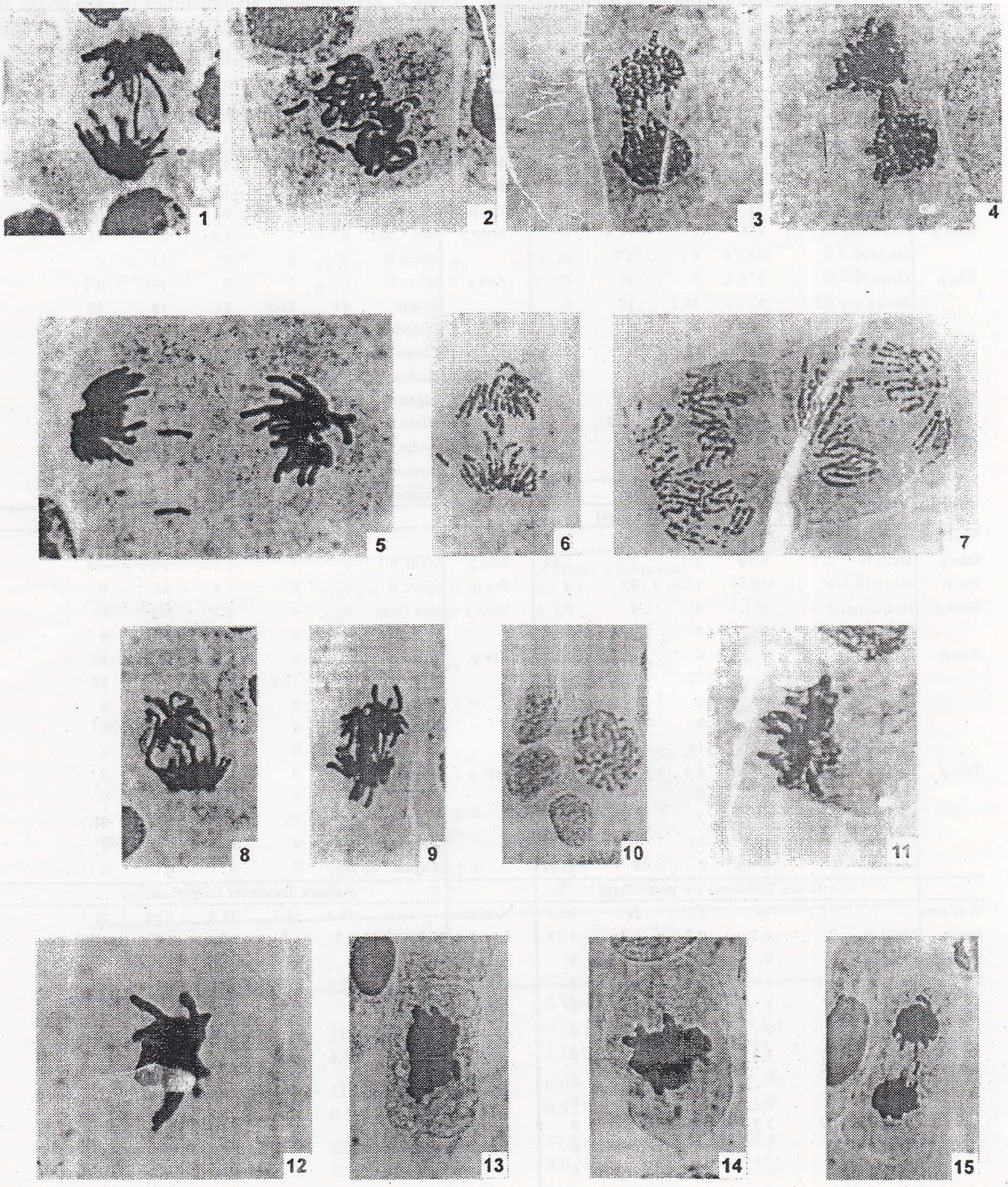

Fig. - 1 to 9 Chromosome bridges and fragments.
1. Anaphase with single bridge; 2 & 3. Anaphase showing many bridges with achromatic lesions
4, 5 & 6. Anaphase with more bridges; 7 & 8. Lagging and fragments in anaphase
9. Metaphase with fragments & clumping.

Fig. - 10.Pycnosis in prophase.

Fig. - 11- 15. Stickiness of chromosome in metaphase.

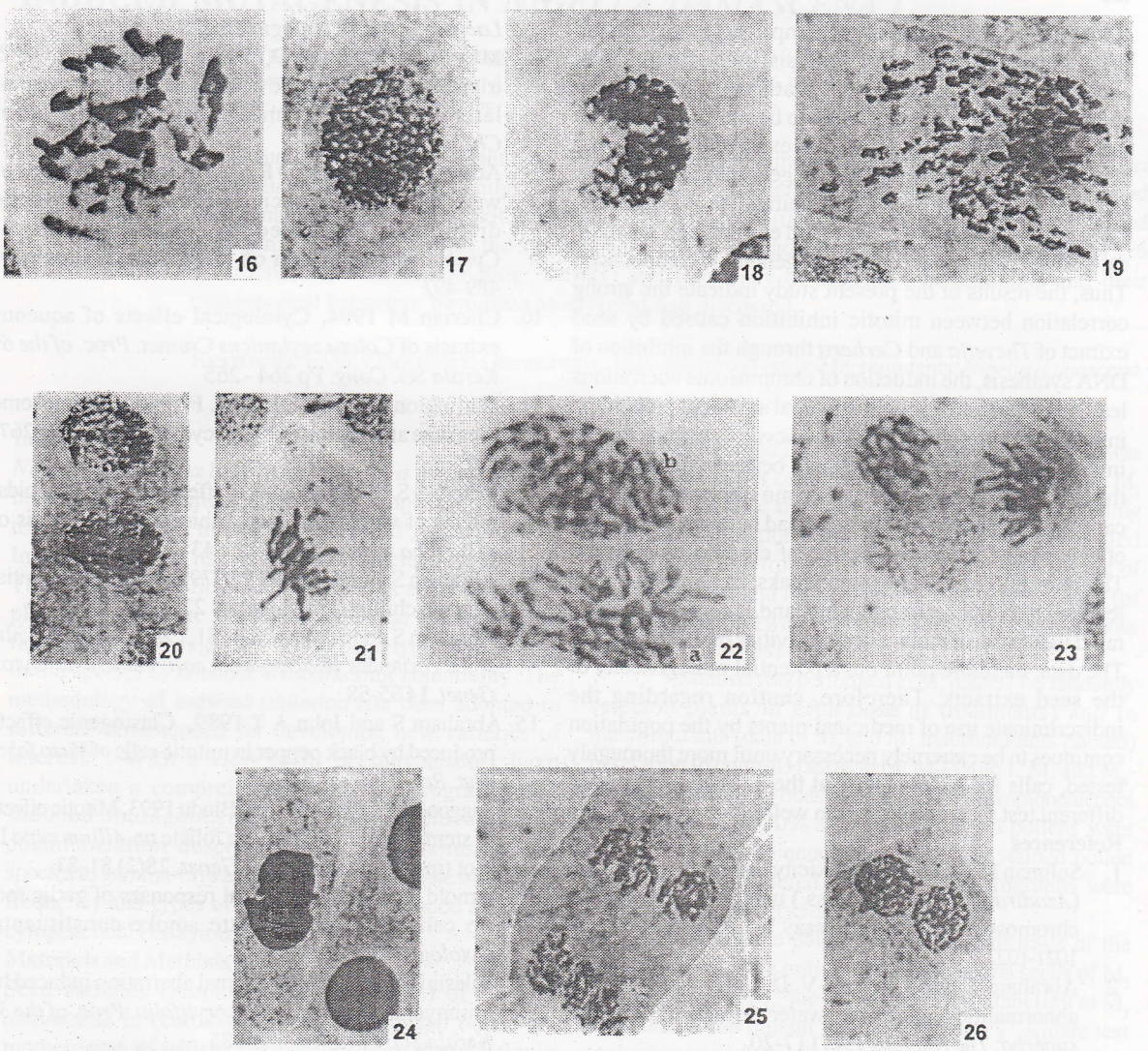

Fig. – 16, 17 & 18. Dissolution of chromosome material in prophase and interphase. Fig. – 19 & 20. Nuclear erosion in prophase and metaphase with gaps. Fig. – 21. Mis congression in metaphase. Fig. – 22 a & b. Ball metaphase Fig. – 23 & 24.Tropokinesis. Fig. – 25. Multipolar anaphase. Fig. – 26. Binucleated cell.

metaphase where the whole chromosome set are not properly arranged at the spindle equator may be due to the intercalary deletion of centromeres or inactivation of centromeres by the action of mutagenic chemical present in the extract[19] (Fig. 21). Ball metaphase characteristically clumped chromosomes was also present followed by either a complete denaturation of the cell or a stage resemble interphase (Fig. 22a & b). Shifting of poles of mitotic apparatus and the direction of spindle axis leads to tropokinesis (Fig. 23, 24). Multipolar anaphase was observed in treated root cells leads to micronuclei (Fig.25). Micronuclei represent a dominant type of anomalous produced by radiations and were recorded in interphase as well as in different mitotic stages. It was considered an indication of true mutation effect[20]. Polyploidy was observed in treated cells. The bi and multinucleated cells observed with this investigation might be due to the suppression of phragmoplast formation in early telophase by the extract treatment[21] (Fig.26). The suppression of mitotic activities was often used for tracing the

cytotoxicity[22]. This is usually accompanied by an increase in the fraction of cells with C- mitosis, multi groups, stick and abnormal chromosome orientation[21]. In the present study, a decrease in MI was found to be significant with an increase the concentrations of seed extract. Chromosomal aberrations occur probably due to genotoxic compounds leads to cause both DNA and spindle protein genetic damage. *Thevetia* and *Cerbera* seed extracts contain toxic cardiac glycosides may function as genotoxic compounds[4]. Thus, the results of the present study indicate the strong correlation between mitotic inhibition caused by seed extract of *Thevetia* and *Cerbera* through the inhibition of DNA synthesis, the induction of chromosome aberrations leading to the loss of genetic material and most probably it interfere with protein synthesis, however, this needs further investigation. A strong correlation between the ability of the chemical to cause chromosome aberration and its capacity to induce point mutation had been reported[8] such effects should involve an action of chromosomal DNA. The induction of chromosome breaks, in particular, may lead to structural rearrangements and is regarded to be a rapid indication of mutagenetic activities of their inducers. The data, therefore point out a potential mutagenecity of the seed extracts. Therefore, caution regarding the indiscriminate use of medicinal plants by the population continues to be extremely necessary until more thoroughly tested, calls for a closer look at the genotoxic effects in different test systems for human welfare.

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