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Effects of some popular medicinal plants on *Mus musculus*

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Abstract — *Mimosa pudica, Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major* are widely used in traditional medicine. In an attempt to determine whether aqueous extracts of the plants interact with genetic material, mammalian cytogenetic assay was used. Mice which received intraperitonial injections of different doses of the extract of *Mimosa pudica* showed dose dependent increase in chromosomal aberrations, SC damages and micronucleus frequency, while the other plant extracts did not induce significantly higher frequency of chromosome aberrations.

Key words: clastogenic, cytogenetic assay, genotoxicity, micronucleus, synaptonemal complex.

INTRODUCTION

Herbal medicines are being used by about 80% of the world population, primarily in developing countries for routine health care and are also entering the therapeutics in the developed countries (Kamboj 2000). These escape toxicity testing before they are marketed as traditional medicines due to inadequate drug laws. Yet many reports reveal that drugs of plant origin are not free from toxic effects. Hepatic failure and even death following ingestion of herbal medicine have been reported (Dicken *et al.* 1994). Traditional eye medicines have been linked to childhood blindness in Nigeria (Harries and Cullinan 1994). Death of 15 persons in USA has been ascribed to a herbal medicine for impotency (Josefson 1996). Several medicinal plants are mutagenic, clastogenic and carcinogenic (Nandi *et al.* 1998).

Mammalian *in vivo* tests have several advantages over *in vitro* tests because the metabolic activation and detoxification of the chemicals in the intact animal are closer to the human system. *In vivo* mouse chromosome assay has been recognized as one of the sensitive methods to test genotoxicity of plant extracts (Chakrabarti 2001). The bone marrow micronucleus (BMM) test is one of the least expensive *in vivo* assays for genotoxic effects (Heddle 1973; Schmid 1976). However, it has limitations that it only detects chromosome breaks or laggards and not even the mature types of break. Therefore, analysis of traditional chromosome aberration was employed in conjunction with BMM test for comprehensive mutagenicity testing. Synaptonemal complex (SC) analysis holds great promise as an *in vivo* mammalian germ cell assay for resolving effects of chemical exposure to the gonads and evaluating the risk of genetic damage (Allen *et al.* 1988; Backer *et al*. 1988).

Five widely used medicinal plants viz., *Mimosa pudica, Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major* were selected for the present study because of their popular medicinal values (Sinha 1996); Prajapati *et al.* 2003). Medicinal uses of these plants are: *M. pudica* – haemorrhoids, urinary infections, dysentery, fever, syphilis, leprosy, veneral disease, insect bite, insomnia, nervousness and piles; *V. trifolia* – sinus, hydrocele, skin disease, glaucoma, opthalmagia, rheumatism and neuralgia; *C. asiatica* – fever, measles, dysentery, constipation, jaundice and furunculosis; *L. aspera* – dyspepsia, verminoxis, arthralgia, chronic skin eruption, cough, intermittent fevers and ulcers; *P. major* – toothace, earace, enuresis, pyorrhea alveolaris, depression, insomnia and pruritis. These plants contain various compounds. *M. pudica* is known to contain tannins (Digrak *et al.* 1999), norepinephrine and crocetin (Prajapati *et al.* 2003). Crocetin is a cytotoxic substance (Jagadeeswaran *et al.* 2000). Vitoeosin-A and vitexicarpin (Alam *et al.* 2002), and luteolin-7-glucoside (Prajapati *et al.* 2003) have been reported in *V. trifolia*. *L. aspera* is known to contain lignans and flavonoids (Sadhu *et al.* 2003), glucoside (Prajapati *et al.* 2003), and

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triterpenoid/steroids (KAMAT and SINGH 1994). *P. major* has been reported to contain triterpene ursolic acid (RINGBOM *et al.* 1998), caffeic acid and cinnamic acid (PRAJAPATI *et al.* 2003), and erucic acid (GUIL *et al.* 1997). Tannins are the water extractable compounds, the mutagenicity of the compound has been reported (PORTO 1999). Crocetin is a cytotoxic substance (JAGADEESWARAN *et al.* 2000). Luteolin-7-glucoside, triterpines betulinic acid and ursolic acid were shown to have cytotoxic activities (KIM *et al.* 2000; GALVEZ *et al.* 2003). Because of the presence of the above compounds in the five medicinal plants some of which are cytotoxic and mutagenic these plants were examined for genotoxicity using mammalian *in vivo* cytogenetic assay. In present study, cytogenetic assay of aqueous extracts of five medicinal plants was carried out in order to determine their clastogenic potentialities using *Mus musculus* bone marrow and germ cells because in folk medicine aqueous extracts of the plants are used (SINHA1996).

MATERIALS AND METHODS

Plant materials were collected from different regions of Manipur and identified based on the vegetative and floral characteristics described in "A hand book of Medicinal plants" (PRAJAPATI *et al.* 2003). Parts of the plants used in traditional medicine (whole plant of *M. pudica,* fruits and leaves of *V. trifolia*, whole plant of *C. asiatica,* leaves and flowers of *L. aspera* and whole plant of *P. major*) were air dried, powdered and extracted with boiling distilled water using Soxhlet apparatus for 3-4 hours, and then filtered through Whatman no. 4, stored at 4°C and used within 24 hours. The concentrations of the extracts were expressed as percentage of dry weights of the plants in water. Aqueous extracts were tested because decoctions of these plants with water only are used in traditional medicine (SINHA1996) and the primary aim of this study was to determine whether the chemicals present in the preparations of these plants used in traditional medicine interact with genetic material. Inbred Swiss albino mice originated from a single parental pair of sibs were maintained in natural environmental condition with free access food (gram, wheat, milk and bread) and water. Permission of the University Ethics Committee was obtained for animal experiments and the Committee's guidelines for human treatment of animals were strictly followed. Mice weighing about 25 g and 10-12 weeks old were used. Maximum tolerated doses of the plant extracts were determined by range test. LD^{50} doses were serially diluted with distilled water until LD^{o} was achieved. LD^{o} was taken as MTD. Animals were treated through intraperitonial injections with MTD and less than MTD of plant extracts. The treatment of same dose of distilled water served as negative control and EMS at the dose of 24 mg/100 g body weight dissolved in 1ml distilled water served as positive control. Each treatment and control group consisted of 5 animals. The treatment protocol is given in Table 1. Animals were sacrificed after 24 hours of treatment and cytological preparations were made. Metaphase chromosomes were prepared from bone marrow cells using standard colchicines - hypotonic – spreading – air drying technique. Synaptonemal complexes (SC) were prepared from spermatocytes following the method of BHAGIRATH and KUNDU (1985). Briefly, one drop of testicular cell suspension in RPMI – 1640 was spread over a large drop of 0.2 M sucrose on a clean slide for 30 seconds, slides were fixed in 4% paraformaldehyde for 5 minutes, washed in 0.2% photoflow for 30 seconds, dried and stained with 70% silver nitrate. SC damages were scored according to ALLEN *et al.* (1998) and BACKER *et al.* (1988). Micronuclei were prepared from bone marrow cells following the method of ROMAGNA and STANIFORTH (1989). Briefly 2-3 drops of bone marrow cell suspension in RPMI-1640 and FCS were put on a clean slide and drawn into a smear with another slide, air dried, fixed in methanol and stored overnight in refrigerator and stained with Giemsa which was filtered through 0.2 µ syringe filter. One slide was prepared from each animal and 1000 PCE were scored from each slide as recommended by two IPCS collaboration studies (ASHBY *et al.* 1983). The results from treated animals were compared with those from control animals using Z-test.

RESULTS

Table 2 presents data on the different types of traditional chromosome aberrations induced by the plant extracts. Representative types of traditional chromosomal aberrations are shown in Figures 1-6. More than 5000 metaphases from five animals were examined for each group. Total number of aberrations of all types were added. Centric fission and centric fusion were the predominant types of abnormality observed in all the treatments. Chromatid break, isochromatid

Table 1 — The experimental protocol for the treatment of animals.

Plant (part)	Treatment		Treatment period	No. of animals treated
	Volume in ml/100g body weight (Concentration)			
	MTD	Half of the MTD		
M. pudica (Whole plant)	1.2 (15%)*	0.6	24hrs	5
V. trifolia (Fruit & leaves)	1.6 (15%)*	0.8	24hrs	5
C. asiatica (Whole plant)	2.4 (15%)*	1.2	24hrs	5
L. aspera (leaves & flower)	2.4 (15%)*	1.2	24hrs	5
P. major (Whole plant)	1.6 (15%)*	0.8	24hrs	5

* Extract concentration expressed as plant material dry weight/100 ml distilled water. Extracts of different weights of each plant material were subjected to range test for determining MTD. Figures in parenthesis indicate concentrations that provided maximum treatment volume of 0.6 ml/animal in MTD range tests.

Table 2 — Chromosomal aberrations in bone marrow cells of mice treated with EMS, distilled water and the plant extracts.

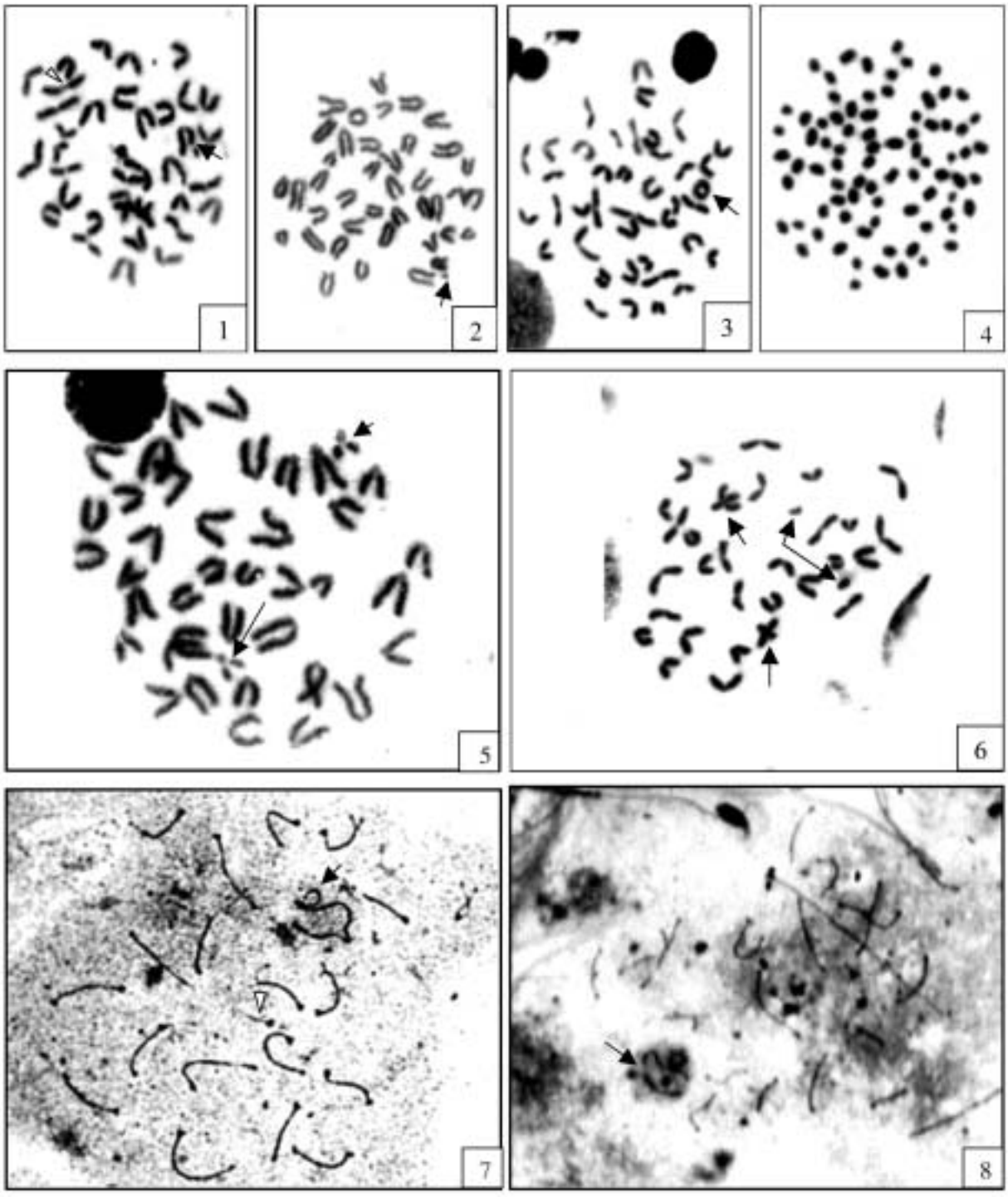
Treatment	No. of meta.	Centro. gap	Centric fusion	Centric fission	Chrom. break	Isochro. break	Trans.	Attenu	Ring Chromo	All dot Like	Frag.	Total damage	% of damage ± SE
EMS 24 mg/100g. b. wt (positive control)	5321	170	367	548	70	58	24	189	29	66	136	1657	31.13*± 0.032
Distilled water (Negative control)	5306	20	55	135	10	0	0	40	8	0	0	268	5.04 ± 0.037
M. pudica													
0.6ml/100g. b. wt	5480	97	183	240	19	8	0	79	15	15	15	671	12.24*± 0.010
1.2ml/100g. b. wt	5295	102	201	263	24	13	0	89	16	20	20	748	14.12*± 0.024
V. trifolia													
0.8ml/100g. b. wt	5286	26	68	132	12	0	0	35	11	0	0	284	5.36 ± 0.039
1.6ml/100g. b. wt	5421	24	75	145	13	0	0	43	12	0	0	312	5.74 ± 0.032
C. asiatica													
1.2ml/100g. b. wt	5450	26	66	145	12	0	0	41	9	0	0	299	5.48 ± 0.023
2.4ml/100g. b. wt	5315	28	75	140	12	0	0	44	12	0	0	311	5.84 ± 0.018
L. aspera													
1.2ml/100g. b. wt	5342	28	72	140	12	0	0	42	12	0	0	306	5.72 ± 0.018
2.4ml/100g. b. wt	5345	30	74	139	12	0	0	44	13	0	0	312	5.82 ± 0.027
P. major													
0.8ml/100g. b. wt	5355	27	58	131	10	0	0	38	10	0	0	274	5.11 ± 0.034
1.6ml/100g. b. wt	5327	27	61	132	12	0	0	39	12	0	0	283	5.31 ± 0.042

Abbreviation: Meta.=Metaphase, Centro.=Centromeric, Chrom.=Chromatid, Trans.=Tnanslocation, Attenu.=Attenuation, Frag.=Fragments, Dam.=Damage. *=P <0.01, SE=Standard Error.

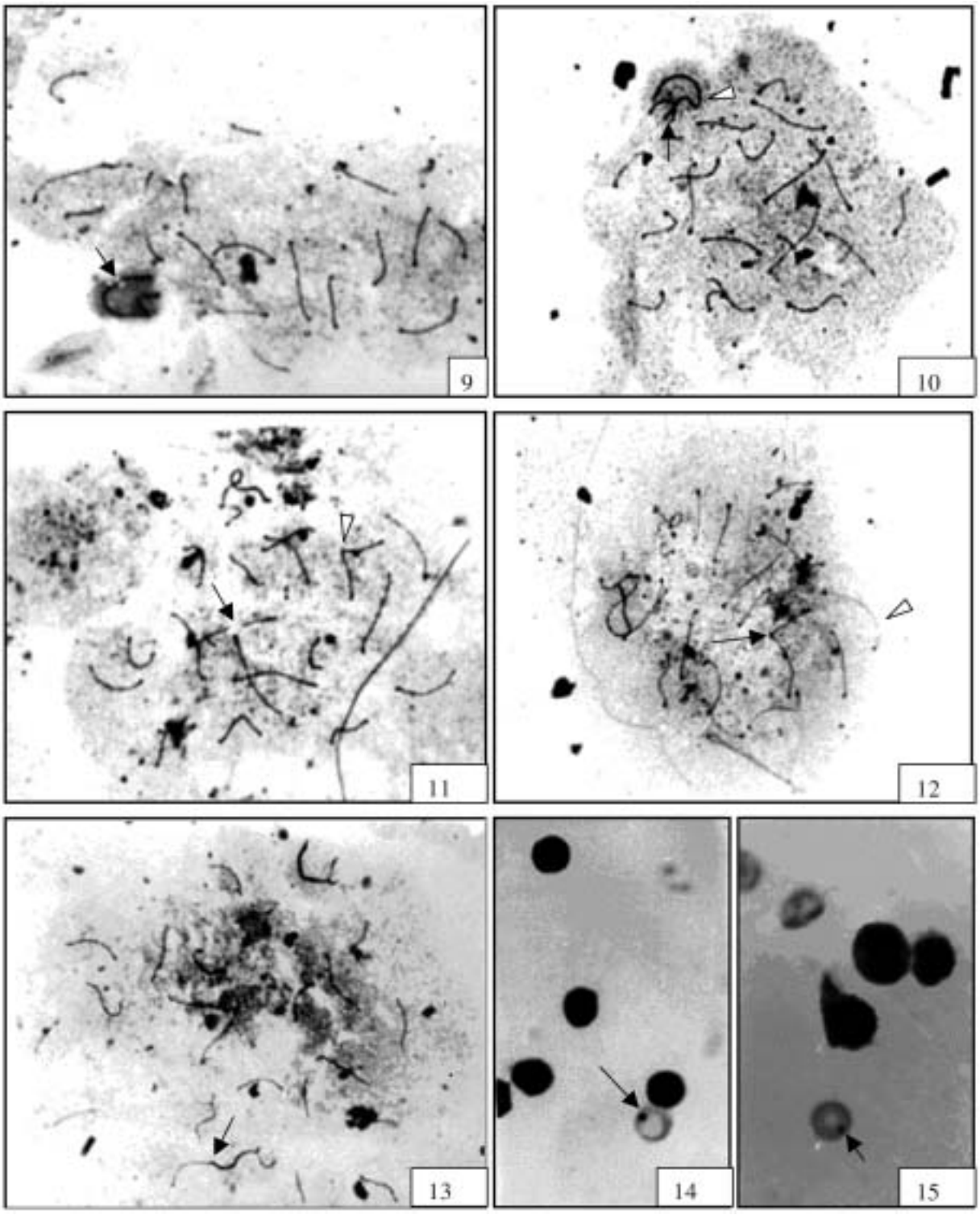
break, ring chromosome, all dot like and fragment were observed at lesser frequency. Frequencies of aberrations in positive and negative control were 31.13% and 5.04% respectively. Frequency of aberrations in animals treated with *M. pudica* extract showed dose dependent increase and was 2-3 times the negative control value (P<0.01) but was about only a half of the positive control value. Frequencies of aberrations in animals treated with *Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major* were similar with control value.

Types of synaptonemal complex damages and their frequencies are shown in Figures 7-13 and Table 3. Types of SC damages are those recommended by ALLEN *et al*. (1988) and BACKER *et al*. (1988). EMS (positive control) induced all types of the recommended damages, while distilled water (negative control) induced only some varying types of damages. Frequency of total damages in mice treated with *M. pudica* extract was about 2-3 times (dose-dependent increase) the negative control value (P<0.01) but only about a half of the positive control value. Mice treated with extracts of *Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major* and distilled water (negative control) showed almost similar values.

MPCE and their frequencies are shown in Figure 15 and Table 4. NCE having red-orange colour (Fig. 14) could be differentiated from bluish coloured PCE (Fig. 15). Proportions of MPCE in mice treated with EMS and *M. pudica* extract were 6 times and 2-3 times of the negative control



Figs. 1-8 — Somatic chromosome aberrations. (1) Chromatid break (solid arrow), centromeric gap (solid arrow head) and attenuation (hollow arrow head); (2) chromosome fragment (solid arrow); (3) ring chromosome (solid arrow); (4) all dot like; (5) isochromatid break (solid arrows); (6) centric fusion (solid arrows) and centric fission (double head arrow). Fig.s 7-8. Synaptonemal complex damages. (7) Y-fold back pairing (solid arrow) and autosome fragment (hollow arrow head); (8) X-break (solid arrow).



Figs. 9-15 — Synaptonemal complex damages. (9) Attenuation in X-element (solid arrow); (10) X-fold back pairing (hollow arrow head) and X-Y separation (solid arrow); (11) autosomal translocation (hollow arrow head) and SC break (solid arrow); (12) autosomal attenuation (hollow arrow head) and autosomal translocation (solid arrow); (13) X-A translocation (solid arrow). Fig. 14. NCE with single micronucleus (solid arrow). Fig. 15. PCE with single micronucleus (solid arrow).

Table 3 — Synaptonemal complex damages in mouse spermatocytes treated with EMS, distilled water and the plant extracts.

Treatment	No of cells scored	Autosome Element Damage							Sex Element Damage								Total dam	% of dam ±SE
		SC break	Frag	MAC	Attn	Asyn	Over all Asyn	Auto-Trans	Attn. in X Elem	Attn. in Y Elem	X-Y Sepn	X-A Tran	Y-A Trans	X-fold back pair	Y-fold Back pair	SC Break in X	SC Break in Y		
EMS 24mg/100g. b. wt (Positive control)	5323	412	305	32	260	17	18	86	32	26	114	34	16	144	15	33	17	1561	29.2*± 0.074
Distilled water (Negative control)	5347	146	0	0	51	0	0	18	0	0	26	17	0	18	0	0	0	276	5.15 ± 0.019
M. pudica 0.6ml/100g.b.wt	5198	209	18	0	106	0	0	31	3	0	45	24	0	63	2	5	0	506	9.72*± 0.019
1.2ml/100g.b. wt	5283	268	33	0	125	0	0	54	9	0	72	24	0	83	4	8	0	680	12.86*± 0.005
V. trifolia 0.8ml/100g. b. wt	5318	156	0	0	52	0	0	14	0	0	19	16	0	19	0	0	0	276	5.18 ± 0.027
1.6ml/100g. b. wt	5195	155	0	0	53	0	0	17	0	0	21	16	0	20	0	0	0	282	5.42 ± 0.037
C. asiatica 1.2ml/100g. b. wt	5234	155	0	0	54	0	0	19	0	0	20	16	0	20	0	0	0	284	5.41 ± 0.049
2.4ml/100g. b. wt	5177	156	0	0	58	0	0	23	0	0	21	20	0	21	0	0	0	299	5.77 ± 0.038
L. aspera 1.2ml/100g. b. wt	5496	165	0	0	55	0	0	24	0	0	27	22	0	26	0	0	0	319	5.79 ± 0.016
2.4ml/100g. b. wt	5540	172	0	0	57	0	0	22	0	0	30	21	0	25	0	0	0	327	5.89 ± 0.011
P. major 0.8ml/100g. b. wt	5229	155	0	0	54	0	0	19	0	0	19	16	0	19	0	0	0	282	5.38 ± 0.050
1.6ml/100g. b. wt	5244	156	0	0	56	0	0	21	0	0	21	17	0	21	0	0	0	292	5.56 ± 0.028

Abrreviation: SC = Synaptonemal complex, Frag = Fragment, MAC = Multi Axial Complex, Attn. = Attenuation, Asyn. = Asynapsis, Auto-trans. = Autosome translocation, Elem. = Element, Sepn. = Separation, Pair. = Pairing, Dam. = Damage, * = P<0.01, SE = Standared Error.

Table 4 — Frequency of MPCE in bone marrow cells of mice treated with EMS, distilled water and the plant extracts.

Treatment	No. of PCE scored	No. of MPCE scored	Ranges of proportion of MPCE (MPCE/ PCE)
EMS 24mg/100g. b.wt (Positive control)	5000	103	0.0206*
Distilled water (Negative control)	5000	17	0.0034
M. pudica			
0.6ml/100g.b.wt	5000	45	0.009*
1.2ml/100g.b.wt	5000	53	0.0106*
V. trifolia			
0.8ml/100g.b.wt	5000	24	0.0048
1.6ml/100g. b.wt	5000	26	0.0052
C. asiatica			
1.2ml/100g.b.wt	5000	23	0.0046
2.4ml/100g. b.wt	5000	25	0.005
L. aspera			
1.2ml/100g.b.wt	5000	22	0.0044
2.4ml/100g. b.wt	5000	23	0.0046
P. major			
0.8ml/100g. b.wt	5000	21	0.0042
1.6ml/100g. b.wt	5000	22	0.0044

* = P<0.01

value respectively (P<0.01). There was no significant difference among treatments with distilled water, extracts of *Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major.*

DISCUSSION

In present study, *Mimosa pudica* extract showed positive results in all three clastogenic testing protocols based on traditional chromosome aberration analysis, micronucleus assay and SC damages analysis. It is recommended that a particular agent proves mutagenic when it shows the mutagenicity at least in more than one test protocols (Bochkov *et al.* 1976; Sharma 1984; Sarkar and Manna 1989). Positive results of mutagenicity for *M. pudica* extract in all the test protocols evidently indicate that *M. pudica* extract contain chemicals which are able to produce different aberrations in somatic and germ cells. The data further revealed that varieties of aberrations and their frequencies had direct relation with the dose of the extract injected.

It is reasonable to suggest that not only the effect of *M. pudica* extract is dose related but also the extract possess genotoxic materials. Regarding the mode of action of the extract, the chemical constituents of the extract might have certain role in the modification of nucleoprotein synthesis in the cells which in turn changes the chromatin organization. Therefore, it is imperative to identify the clastogenic compounds and to explore the methods for its removal.

Although *Vitex trifolia, Centella asiatica, Leucas aspera* and *Plantago major* showed negative results in all the genotoxicity testing protocols, further specific and reliable mutagenicity tests need to be done as there are cases of point mutation, frame shift mutation which escape detection by methods employed in the present study. Mutagenicity of certain medicinal plants observed in the earlier studies by other investigators (Nandi *et al.* 1998) compels the necessity of using medicinal plants with specific guidelines, which have a sound basis and relevance to the population concerned.

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