International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor- 5.46, Volume 5, Issue 3, March 2018
Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

GENOTOXIC EFFECTS OF SOME POPULAR MEDICINAL PLANTS ON *MUS MUSCULUS*

Kshetrimayum Guneshwor
Department of Zoology, Ideal Girls' College, Akampat, Imphal-East, India

Adhikarimayum Haripyaree
Department of Botany, Imphal College, Imphal, India

ABSTRACT

Nerium odurum, Kaempferia galanga and Nyctanthes arbortristis 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 Nerium odurum showed dose dependent increase in chromosomal aberrations, SC damages and micronucleus frequency, while Kaempferia galanga and Nyctanthes arbortristis did not induce significantly higher frequency of chromosome aberrations.

Key word: 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).

Three widely used medicinal plants viz., *Nerium odurum, Kaempferia galanga* and *Nyctanthes arbortristis* were selected for the present study because of their popular medicinal values (PRAJAPATI *et al.* 2003; SINHA 1996). Medicinal uses of these plants are : *N. odurum* – haemorrhage, cutanous eruptions, opthalmia, cardiotonic, ulcerous agent and ground root bark is a potent abortifacients and is frequently fatal to the mother; *K. galanga*- cough, stomach and gastric complaints; *N. arbortristis* – chronic fever, liver and spleen complaints, rheumatism. These plants contain various compounds. Glucoside and odoroside B have been isolated from the roots of *N. odurum* (HUG *et al.* 1998). *K. galanga* rhizome contains flavonoids, sesquiterpenoids and cinnamic acid (PANJI 1993). Terpenoids, saponins and iridoid glycosides are present in the leaves of *N. arbortristis* (JAIN and MUKHOPADHYAY 1996). Flavonoids are water extractable organic compounds that are mutagenic in *Salmonella* microsome assay in the strains TA 100 and TA 98 (PORTO 1999). Because of the presence of the above compounds in the three 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 three 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 (root of *N. odorum,* Rhizome of *K. galanga*, leaves of *N. arbortristis*) 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 4ºC 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 to 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 break, ring chromosome, all dot like and fragment were observed at lesser frequency. Frequencies of aberrations in positive and negative control were 30.39% and 4.31% respectively. Frequency of aberrations in animals treated with *N. odorum* 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 *K. galanga* and *N. arbortristis* were similar with control value.

Types of synaptonemal complex damages and their frequencies are shown in figures 7 to 15 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 *N. odorum* 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 *K. galanga* and *N. arbortristis* and distilled water (negative control) showed almost similar values.

MPCE and their frequencies are shown in figures 16 to 17 and Table 4. NCE having red–orange colour (figure 17) could be differentiated from bluish coloured PCE (figure 16). Proportions of MPCE in mice treated with EMS and *N. odorum* extract were 6 times and 2-3 times of the negative control value respectively ($P<0.01$). There was no significant difference among treatments with distilled water, extracts of *K. galanga* and *N. arbortristis.*

Discussion

In present study, *Nerium odorum* 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 (SARKAR and MANNA 1989; BOCHKOV *et al.* 1976; SHARMA 1984). Positive results of mutagenicity for *N. odorum* extract in all the test protocols evidently indicate that *N. odorum* 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 *N. odorum* 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 *K. galanga* and *N. arbortristis* 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.

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		
N. odorum (Root)	2.4 (15%)*	1.2	24 hrs	5
K. galanga (Rhizome)	5.2 (15%)*	2.6	24hrs	5
N. arbortritis (Leaves)	4.8 (15%)*	2.4	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 1.3 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)	5323	189	368	544	64	52	19	162	26	59	135	1618	30.39*± 0.032
Distilled water (Negative control)	5331	33	47	125	6	0	0	15	4	0	0	230	4.31 ± 0.037
N.odorum													
1.2ml/100g. b. wt	5454	77	180	231	16	5	0	99	9	11	8	628	11.51*±
2.4ml/100g. b. wt	5382	93	211	269	21	9	0	112	11	14	16	756	0.010
K.galanga													
2.6ml/100g. b. wt	5283	35	58	137	8	0	0	23	8	0	0	269	5.09 ± 0.039
5.2ml/100g. b. wt	5312	29	62	147	6	0	0	27	7	0	0	280	5.27 ±
N.arbortristis													
2.4ml/100g. b. wt	5445	39	63	129	9	0	0	19	6	0	0	265	4.86 ±
4.8ml/100g. b. wt	5389	35	59	139	7	0	0	26	9	0	0	275	0.023

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

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

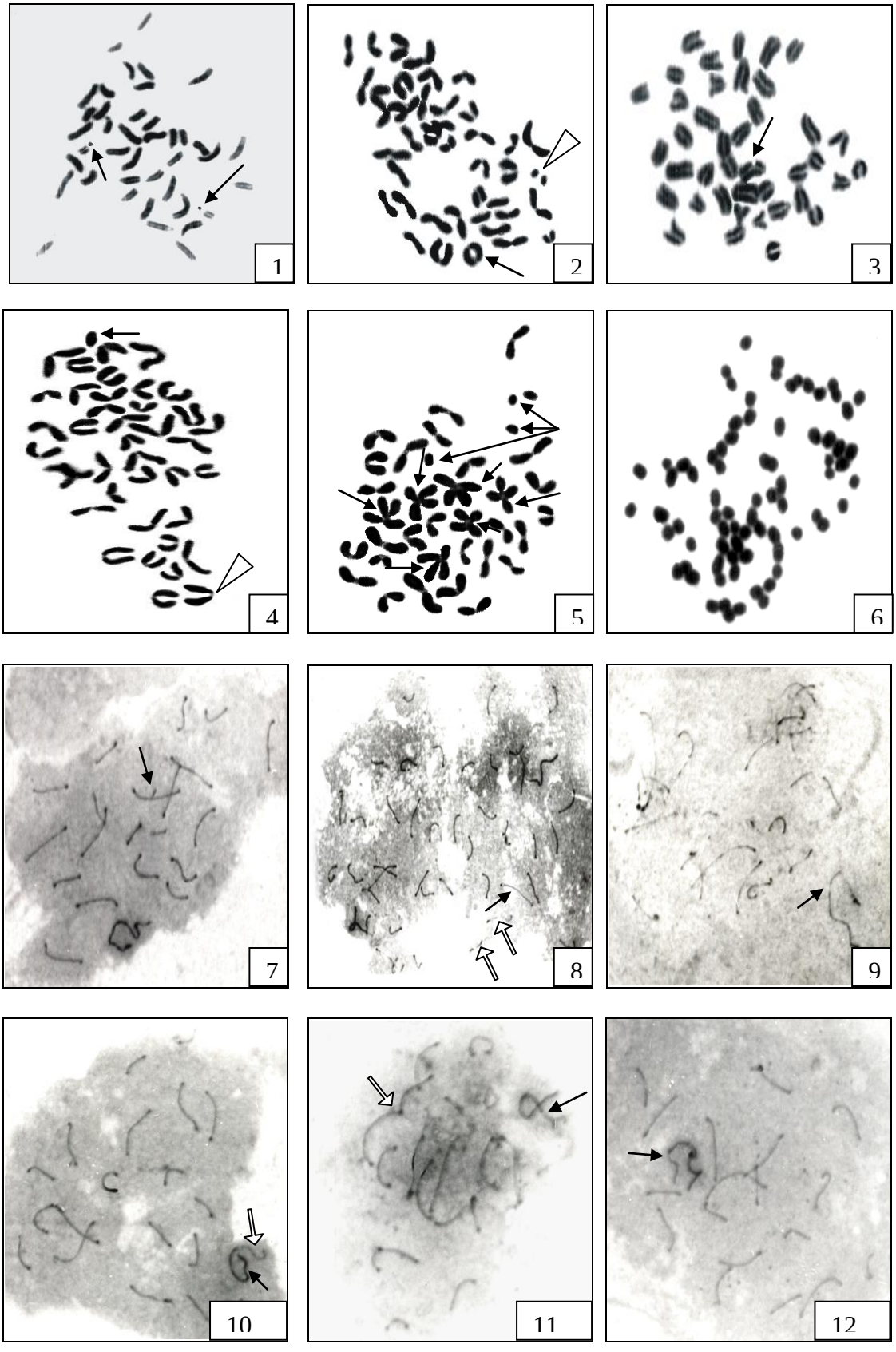
Treatment	No.of cells scored	Autosome Element Damage							Sex Element Damage								Total dam	% of dam. ±SE
		SC break	Frag	MAC	Attn	Asyn	Overall Asyn	Auto-Trans	Attn. in X Elem	Attn. in Y Elem	X-Y Sepn	X-A Trans	Y-A Trans	X-fold back pair	Y-fold Back pair	SC Break in X	SC Break in Y		
EMS 240mg/100g. b. wt (Positive control)	5362	419	289	31	253	13	9	78	29	25	103	27	13	141	13	23	15	1481	27.62*± 0.074
Distilled water (Negative control)	5337	139	0	0	43	0	0	7	0	0	14	8	0	9	0	0	0	220	4.12 ± 0.019
N .odorum																			
1.2ml/100g.b.wt	5245	213	7	0	97	0	0	31	4	0	37	16	0	59	3	6	3	476	9.07*± 0.019
2.4ml/100g.b. wt	5198	263	25	0	112	0	0	46	11	0	67	17	0	71	5	7	4	628	12.08*± 0.005
K. galanga																			
2.6ml/100g. b. wt	5385	163	0	0	51	0	0	17	0	0	21	19	0	15	0	0	0	286	5.31 ± 0.027
5.2ml/100g. b. wt	5364	167	0	0	58	0	0	21	0	0	25	21	0	17	0	0	0	307	5.72 ± 0.037
N.arbortristis																			
2.4ml/100g. b. wt	5217	135	0	0	37	0	0	12	0	0	11	15	0	12	0	0	0	232	4.44 ± 0.049
4.8ml/100g. b. wt	5246	147	0	0	41	0	0	25	0	0	14	17	0	14	0	0	0	258	4.91 ± 0.038

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	5000	101	0.0202*
(Positive control)			
Distilled water	5000	19	0.0038
(Negative control)			
N. odorum			
1.2ml/100g.b.wt	5000	42	0.0084*
2.4ml/100g.b.wt	5000	51	0.0102*
K.galanga			
2.6ml/100g.b.wt	5000	26	0.0052
5.2ml/100g. b.wt	5000	28	0.0056
N. arbortristis			
2.4ml/100g.b.wt	5000	24	0.0048
4.8ml/100g. b.wt	5000	26	0.0052

*= $P<0.01$


1
2
3
4
5
6
7
8
9
10
11
12

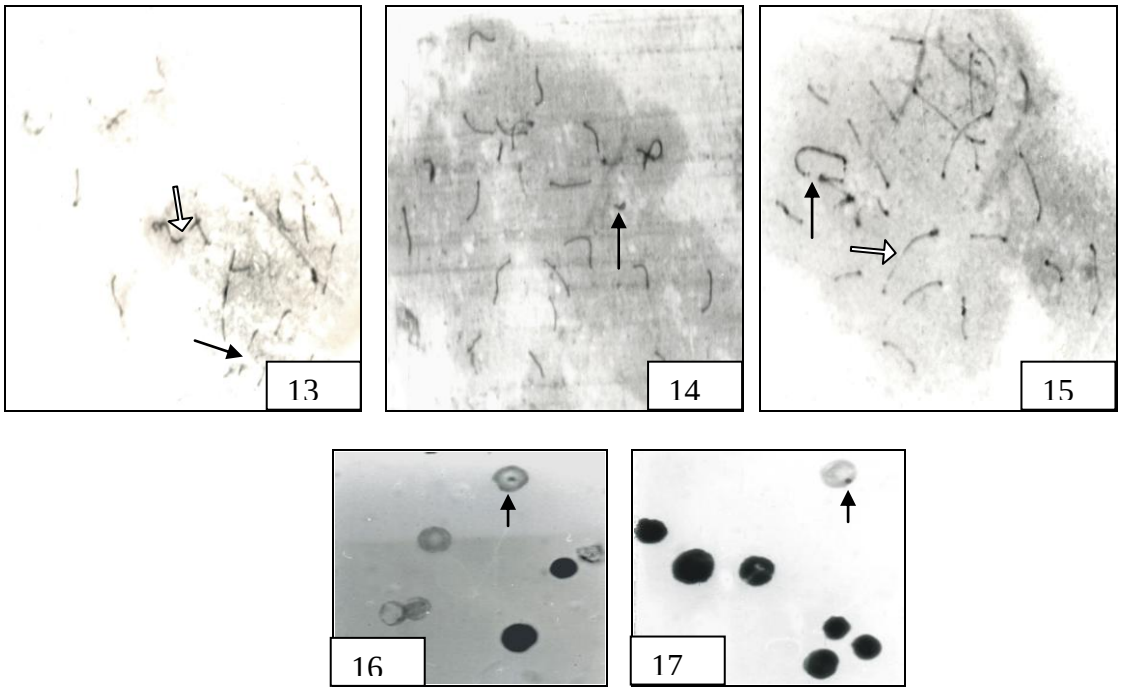


Figure 1-6. Somatic chromosome aberrations. (1) Isochromatid break (solid arrows); (2) Ring chromosome (solid arrow) and Centric fission (hollow arrow); (3) Chromatid break (solid arrow); (4) Centromeric gap (hollow arrow) and a fragment (solid arrow); (5) Centric fission (solid three head arrow) and Centric fusion (solid arrows); and (6)All dot like.

Figure 7-15. Synaptonemal complex damages. (7) Autosome fragment (solid arrow); (8) Autosomal attenuation (solid arrow) and SC break (hollow arrow); (9) Attenuation in X-element (solid arrow); (10) X-fold back pairing (solid arrow) and Y-fold back pairing (hollow arrow); (11) Autosome translocation (hollow arrow) and Attenuation in X-element (solid arrow); (12) X-A translocation (solid arrow); (13) X-break (hollow arrow) and SC break (solid arrow); (14) Autosome fragment (solid arrow) and (15) X-Y separation (solid arrow) and SC break (hollow arrow).

Figure 16. PCE with single micronucleus (solid arrow).

Figure 17. NCE with single micronucleus (solid arrow).

References

ALLEN J.W., GIBSON J.B., POORMAN P.A., BACKER L.C. and MOSES M.J.,1988 — *Synaptonemal complex damages induced by clastogenic and antimitotic chemical: implications for non-disjunction and aneuploidy.* Mut. Res., 301:313-324.

ASHBY J., SERRES DE F.J., DRAPPER M.H., ISHIDATE J.K., MATTER B.I. and SHELBY M.D., 1983 — *The two IPCS collaboration studies on short-term test system of genotoxicity and carcinogenicity*. Mut. Res., 109: 123-126.

BACKER L.C., GIBSON J.B., MOSES M.J. and ALLEN J.W., 1988 — *Synaptonemal complex damage in relation to meiotic chromosome aberrations after exposure of male mice to cyclophosphamide.* Mut. Res., 203: 317.

BHAGIRATH TH. and KUNDU S.C., 1985 — *Effects of male contraceptive agent gossypol on bone-marrow chromosomes of the male rat.* Cytogenet. Cell Genet., 39: 228-230.

BOCHKOV N.P., SRAM R.J., KULESHOV N.P. and ZHURKOV V.S., 1976 — *System for the evaluation of the risk from chemical mutagens: basic principles and practical recommendations.* Mut. Res., 38: 191-202.

CHAKRABARTI C.S., 2001 — *Genotoxic effects of Euporatorium extract on the bone-marrow chromosomes of Rat (Rattus norvegicus).* Proc. Zool. Soc. Calcutta., 54: 22-26.

DICKENS P., TAI Y.T., BUT P.P., TOMLISON B., NG H.K. and YAN K.W., 1994 — *Toxicity of complementary therapies: An Eastern perspective.* Forensic Sci. Int., 67: 55-58.

HARRIES A.D. and CULLINAN T., 1994 — *Herbis et orbis: the dangers of traditional eye medicines*. Lancet., 344: 1588.

HEDDLE J.A., 1973 — *A rapid in vivo test for chromosomal damage*. Mut. Res., 18:187-190.

JOSEFSON D., 1996 — *Herbal stimulant causes US deaths*. Br. Med. J., 312: 1378-1379.

KAMBOJ V. P., 2000 —*Herbal Medicine.* Curr. Sci., 78: 35-37.

NANDI P., TALUKDAR G. and SHARMA A., 1998 — *Plants against cancer-some aspects*. Nucleus., 41: 53-86.

PORTO ALEGRE R.S., 1999 — *Mutagenicity of medicinal plant extracts in Salmonella/ microsome assay.* Phytotherapy Res., 13: 397-400.

PRAJAPATI N.D., PUROHIT S.S., SHARMA A.K. and KUMAR T., 2003 — *A hand book of Medicinal Plants- A Complete Source Book.* 1st Edn. Agrobios, India.

ROMAGNA F. and STANIFORTH C.D., 1989 — *Automated bone-marrow micronucleus test*. Mut. Res., 231: 91-104.

SARKAR A.K. and MANNA G.K., 1989 — *Mutagenic potentiality of the nitrogen fixing bacterium Beijerinckia acida tested in mice.* In: Perspectives in Cytology and Genetics, (G. K. Manna and Sinha, eds)., 6: 633-639.

SCHMID W., 1976 — *Chemical Mutagens, Principle and Method for Detection*. (Ed. Holaender) Plenum press New York., 4: 31.

SHARMA A., 1984 — *In: Indian Nat. Sci. Acad. Golden Jubilee publication*. Perspective report., 61.

SINHA S.C., 1996 — *Medicinal Plants of Manipur*. Manipur Association for Science and Society, Imphal.