

Assessment of Genotoxic Effect of *Paris polyphylla*

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Abstract

The rhizome of *Paris polyphylla* Smith is used in number of health problems by the resident people of Manipur and connecting states. In the present study traditional chromosome aberration analysis, micronucleus assay and SC damages analysis are the three testing protocols of the cytogenetically assay to study whether aqueous extract of the plant interrelate with genetic material. Intraperitoneal injections of 3.6 ml/100g body weight (MTD) and 1.8 ml/100g body weight (less than MTD) of the aqueous extract to the animals have no significant higher frequencies. Animals received doses of distilled water equivalent to that of plant extract was recognized as negative control, while EMS at the dose of 240 mg Kg⁻¹ body weight dissolved in 1ml distilled water was recognized as the positive control. The observation of no significant higher frequencies of chromosome aberrations, SC damages and micronucleus indicating negative results of mutagenicity of the plant extract in all the test protocols.

Key words: Mutagenicity, genotoxicity, clastogenicity, cytogenetic assay, LD⁵⁰, maximum tolerated dose, less maximum tolerated dose, intraperitoneal.

Introduction

The earliest recorded evidence of using herbal medicine in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. Medicinal plants have featured in folklore and tradition throughout the world. Herbal medicines are the synthesis of therapeutic experiences of generation of practicing physician of indigenous system of medicines. Indo-Burma biodiversity region of North- East India is a distinct part and is one of the hottest with 6th rank among the 25 mega biodiversity hotspots (MYERS, 1988; MYERS *et al.*, 2000). Manipur which belongs to north-east India bordering Myanmar has explored many species and varieties of medicinal plants. This state is a natural habitat of one of the

most useful and costly herb called *Paris polyphylla* Smith. The rhizome of the herb is used in number of health problems like anti-inflammatory, cancer and bleeding in other parts of world (IUCN, 2004; LEE *et al.*, 2005; SUN *et al.*, 2007; FU, 2007; GUANGLIA *et al.*, 2013; MAN *et al.*, 2017; SONGSONG *et al.*, 2017).

According to World Health Organization (WHO) approximately 80% of world's population in developing countries depends on traditional medicines for primary health care (WHO, 2016). Since inadequate drug laws traditional medicines escape toxicity testing before they are marketed. Drugs of plant source are not unrestricted from toxic effects were reported yet. Ingestion of herbal medicine leads to hepatic failure and even following death have been reported (DICKEN *et al.* 1994). Childhood blindness in Nigeria have been reported due to traditional eye medicines (HARRIES and CULLINAN 1994). A herbal medicine for impotency has been ascribed to 15 persons death in USA (JOSEFSON 1996). Mutagenic, clastogenic and carcinogenic activities were shown by several medicinal plants (NANDI *et al.* 1998).

In vivo mammalian 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. One of the delicate procedures to test genotoxicity of plant extract has been recognized *in vivo* mouse chromosome assay (CHAKRABARTI 2001) and also one of the smallest exclusive *in vivo* assays for genotoxic effects by the (BMM) bone marrow micronucleus test (HEDDLE 1973; SCHMID 1976). The BMM test system can only detects chromosome breaks or laggards and not steady the mature types of break. This leads to analysis of traditional chromosome aberration in conjunction with BMM test for comprehensive mutagenicity testing. Mammalian *in vivo* germ cell assay for determining effects of chemical overview to the gonads and assessing the hazard of genetic damage by the Synaptonemal complex (SC) analysis which holds great promise (ALLEN *et al.* 1988; BACKER *et al.* 1988).

The chemical constituents of *P. polyphylla* are mainly paris saponins such as diosgenins and polyphyllins (WU *et al.*, 2004). Paris saponin VII suppressed the growth of human cervical cancer Hela cells (ZHANG *et al.*, 2014). Paris polyphylla extract induces Apoptosis and activates Cancer Suppressor Gene (LI *et al.*, 2012). Cytotoxicity of methanolic extract of rhizomes of *P. polyphylla* was determined by MTT assay on three cancer cell lines: HeLa, HepG2 and PC3 (DAWA *et al.*, 2019). Polyphyllin D and dioscin evade drug resistance and generate Apoptosis in liver cancer HepG2, R-HepG2 cells (CHEUNG *et al.*, 2005; DENG *et al.*, 1999; GAO *et al.*, 2011; LI *et al.*, 2001). Paris saponins I exhibited effective

radiosensitivity against gefitinif resistant lung adenocarcinoma cell line (JIANG *et al.*, 2014). Polyphyllin D is a steroidal saponin which effects in growth inhibition of human breast cancer cells and in Xenograft (LEE *et al.*, 2005). *In vivo* mammalian cytogenetic assay for the genotoxicity testing of the plant extract have to be carried out since many compounds as mentioned above were contained in the extract even some of them are cytotoxic.

Materials and Methods

Collection and identification of plant defined in “A hand book of Medicinal plants” (PRAJAPATI *et al.* 2003). Extraction of parts of the plant was done with boiling distilled water using Soxhlet apparatus with treatment protocol Table 1. In traditional used of medicine plant parts were boiled with water and hence aqueous extract was tested (SINHA 1996) to examine whether the chemicals present in the preparation of this plant interact with genetic material. Inbred Swiss albino mice attaining the weight about 25 g and 10-12 week were sacrificed. Maximum tolerated doses, less than MTD were determined in the MTD range test. Animals received doses of distilled water equivalent to that of plant extract was recognized as negative control, while EMS at the dose of 240 mg Kg⁻¹ body weight dissolved in 1ml distilled water was recognized as the positive control. Bone marrow cells are flashed out from the animals femur bones for metaphase chromosomes study after the treatment of colchicines 24 hours, make the cells hypotonic which are spread and air dried. Spermatocytes are collected for the study of Synaptonemal complexes (SC) damages, BHAGIRATH and KUNDU (1985) and damages were counted, ALLEN *et al.* (1998) and BACKER *et al.* (1988). Bone marrow cells are flashed out for micronuclei preparation, ROMAGNA and STANIFORTH (1989) and scoring of micronuclei was done following the recommendation by two IPCS collaboration studies (ASHBY *et al.* 1983).

Statistical analysis

Statistical analysis of chromosome aberrations, micronuclei analysis and synaptonemal complex damages from the quantitative datas.

Results

Traditional chromosome aberration frequencies and representative types were described in Table 2 and Figs. 1-6. Frequencies of aberration as examined from more than 5000 metaphases in five animals were 30.12% and 4.08% respectively in positive and negative control. Frequencies of aberration in animals treated with the plant extract were similar with negative control value. The treatment of aqueous extract of the plant observed no significant higher frequencies of chromosome aberrations, SC damages and micronucleus indicating negative results of mutagenicity of the plant extract in all the test protocols.

Synaptonemal complex damages types and their frequencies as suggested by ALLEN *et al.* (1988) and BACKER *et al.* (1988) were described in Figures 7-12 and Table 3. All types of the recommended synaptonemal complex damages were induced with the treatment of EMS as positive control, while only some varying types of damages were induced with the treatment of distilled water as negative control. Frequencies of synaptonemal complex damages in animals treated with the plant extract were similar with negative control value showing no significant higher frequency of SC damages with the treatment of aqueous extract of the plant.

MPCE and their frequencies were described in Figure 14 and Table 4. Red–orange coloured NCE (Fig.13) could be differentiated from bluish coloured PCE (Fig.14). There was no significant difference in the proportion of MPCE among treatments with distilled water and plant extract. The treatment of plant extract did not induce significantly higher micronucleus frequency.

Discussion

In present study, aqueous extract of the plant showed no significant higher frequencies of chromosome aberrations, SC damages and micronucleus indicating negative results of mutagenicity of the plant extract in all the test protocols. It is endorsed 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). Additional precise and consistent mutagenicity tests need to be followed meanwhile there are cases of point

mutation, frame shift mutation which outflow recognition by methods working in the present study, even the plant extract showed negative results in all the genotoxicity testing protocols. Mutagenicity of certain medicinal plants detected in the past studies by other investigators (NANDI *et al.* 1998) induces the requirement of using medicinal plants with precise strategies, which have a thorough foundation and significance 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		
<i>P. polyphylla</i> (Rhizome)	3.6(10%)*	1.8	24 hrs	5

* Extract concentration expressed as plant material dry weight/100 ml distilled water. Extracts of maximum treatment volume of 0.9ml/animal in MTD range tests.

Table 2. Chromosomal aberrations in bone marrow cells of mice treated with EMS, distilled water and the *P. polyphylla* extract.

Treatment	No. of Meta.	Centro. Gap	Centric fusion	Centric Fission	Chrom. Break	Isochro. Break	Trans.	Attenu.	Ring Chromo	All dot Like	Frag.	Total Dam.	% of dam. ± SE
EMS													
240mg Kg ⁻¹ . b. wt (positive control)	5311	185	362	541	65	51	15	160	28	61	132	1600	*30.12±0.087
Distilled water (Negative control)	5325	29	45	123	4	0	0	13	3	0	0	217	4.08±0.026
<i>P. polyphylla</i> extract													
0.9ml/100g. b. wt	5376	26	50	124	7	0	0	14	3	0	0	224	4.16±0.027
0.45ml/100g. b. wt	5261	25	49	125	5	0	0	12	4	0	0	215	4.08±0.025

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

Table 3. Synaptonemal complex damages in mouse spermatocytes treated with EMS, distilled water and the *P. polyphylla* extract.

Treatment	No. of cells scored	Autosome Element Damage										Sex Element Damage						Total Dam.	% of dam. \pm SE
		SC break	Frag	MAC	Attn	Asyn	Over all Asyn	Trans	Auto-Trans	Attn. in X Elem	Attn. in Y Elem	X-Y Seprn	X-A Trans	Y-A Trans	X-fold back pair	Y-fold Back in X	SC Break		
EMS 240mg Kg ⁻¹ b. wt (Positive control)	5354	423	294	26	251	13	9	82	26	21	108	29	12	138	11	27	13	1483	*27.69 \pm 0.080
Distilled water (Negative control)	5343	135	0	0	42	0	0	7	0	0	15	7	0	9	0	0	0	215	4.01 \pm 0.023
<i>P. polyphylla</i> extract	5265	134	0	0	39	0	0	8	0	0	15	6	0	11	0	0	0	213	4.04 \pm 0.021
0.9ml/100g. b. wt	5244	135	0	0	38	0	0	7	0	0	14	5	0	12	0	0	0	211	4.02 \pm 0.019

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

Table 4. Frequency of MPCE in bone marrow cells of mice treated with EMS, distilled water and the *P. polyphylla* extract.

Treatment	No. of PCE Scored	No. of MPCE scored	Ranges of proportion of MPCE (MPCE/PCE)
EMS 240mgKg ⁻¹ b. wt (Positive control)	5000	89	*0.0178
Distilled water (Negative control)	5000	9	0.0018
<i>P. polyphylla</i> extract 0.9ml/100g. b. wt 0.45ml/100g. b. wt	5000 5000	8 7	0.0016 0.0114

* = P<0.01



Paris polyphylla

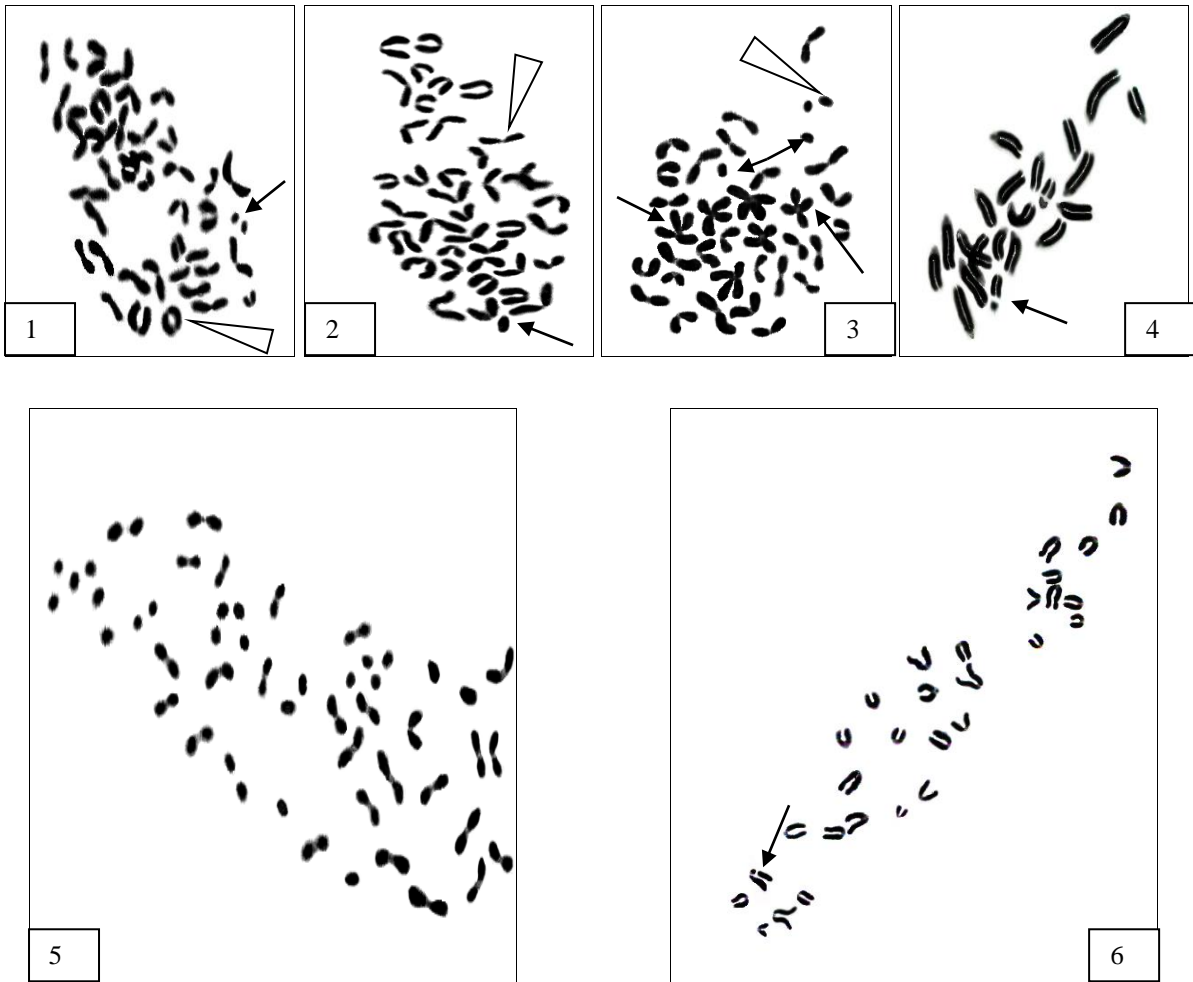


Figure 1-5. Somatic chromosome aberrations. (1) Ring chromosome (hollow arrow) and Centric fission (solid arrow); (2) Centromeric gap (hollow arrow) and Chromosome fragment (solid arrow); (3) Centric fusion (solid arrows), Centric fission (double head arrow and hollow head); (4) All dot like; (5) Isochromatid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

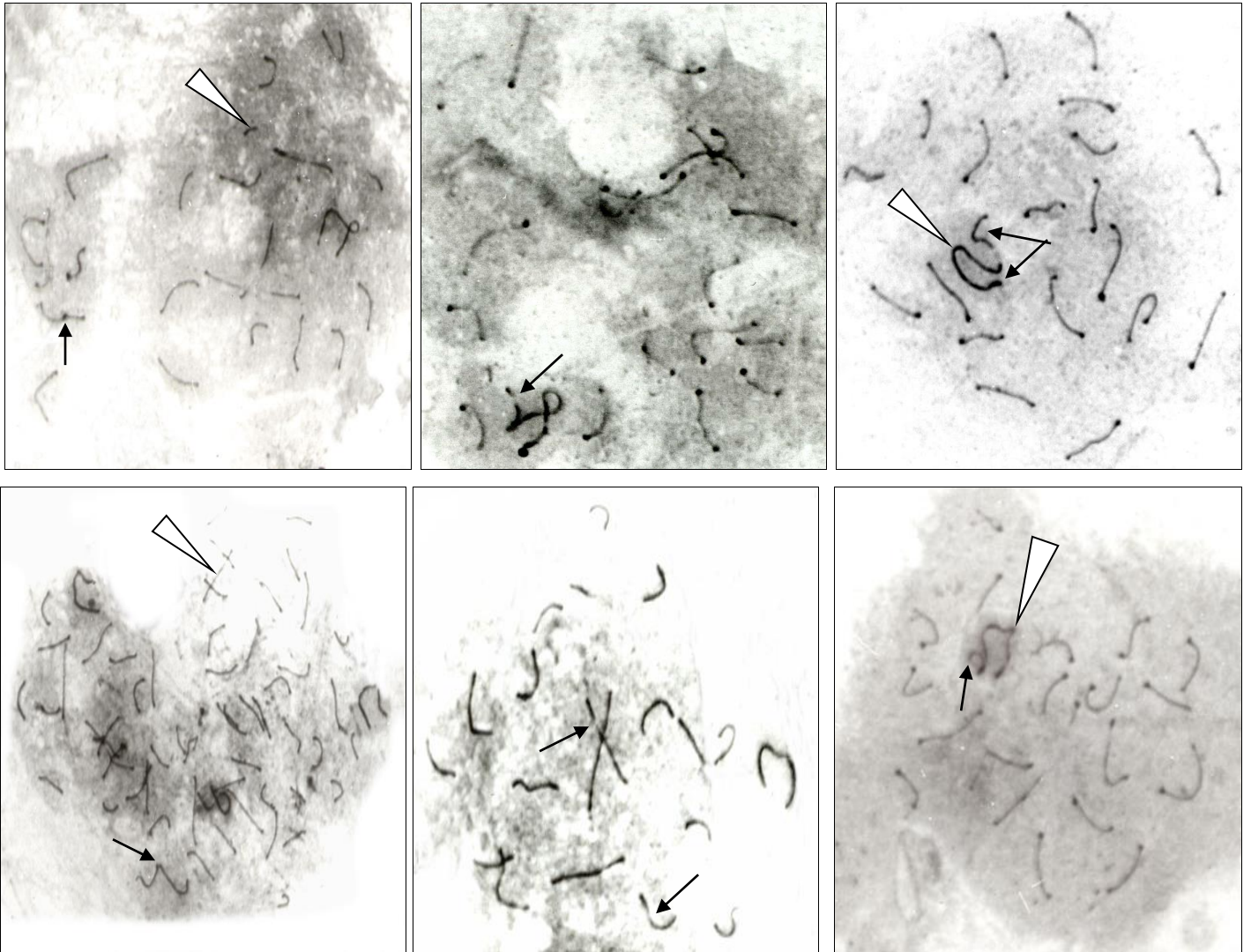


Figure 7-12. Synaptonemal complex damages. (7) Autosome fragment (Hollow arrow) and Autosome translocation (Solid arrow); (8) Y - break (solid arrow); (9) X-Y separation (Double head solid arrow) and X fold back (10) Autosome translocation (solid arrow) and Autosomal attenuation (hollow arrow) ; (11) SC breaks (Solid arrows); (12) Y-fold back (solid arrow) and X-A translocation (hollow arrow).

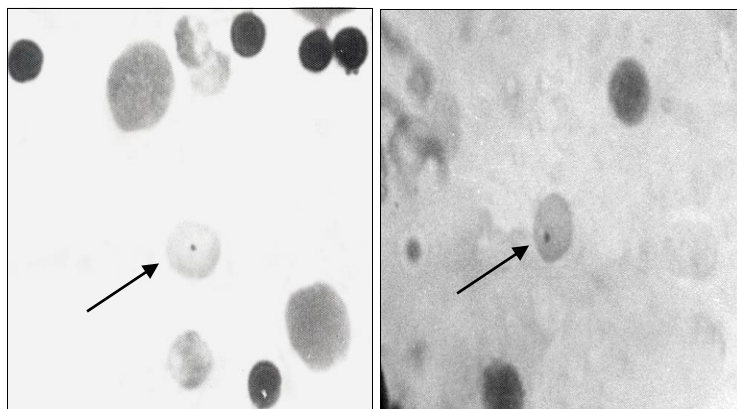


Figure 13. NCE with single micronucleus (solid arrow). Figure 14. PCE with single micronucleus (solid arrow)

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