Ameliorating effect of Barleria lupulina Lindl.extract against γ (gamma)-ray (1.2 Gy) induced mitotic chromosomal aberrations of house musk shrew Suncus murinus

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Abstract— **In the pioneering work Sur PK & Das PK (2012a) , Das PK & Sur PK (2012b), observed that leaf extract of Barleria lupulina (BLPE) has anti-clastogenic, anti-tumor, radio-protective and anti-cancer activities in mice and fish. In the present study we assessed the ameliorating effect of the leaf extract on γ-ray (dose = 1.2Gy) induced mitotic chromosomal aberrations of house musk shrew Suncus murinus. The shrews were divided into three sets i.e. BLPE pre-treated series (SET I), γ-irradiated Control Series (SET II) and BLPE post-treated series (SET III). Cytogenetic studies from the bone marrow cells were carried out by colchicine-citrate-flame-drying technique. Different types of structural chromosomal aberrations were observed and studied. Maximum chromosomal aberrations were observed after 16 hr of γ-irradiation (10.25%) in SET II animals, whereas that for SET I it was 5.28% and for SET III it was 3.16%, in the same time interval. Total aberrations were 5.16%, 6.82% and 3.08% for SET I, SET II and SET III respectively. Therefore BLPE was found to show 54.83% ameliorating effect in shrew Suncus murinus.**

Index Terms— **Barleria lupulina Lindl; γ (gamma)-ray; chromosomal aberrations; shrew bone marrow cells; radiation protection; anti-clastogenic; anti-tumor; anti-cancer.**

I. INTRODUCTION

Cancer is a dreaded threatening disease threatening hundreds of lives all over the globe. Malignant tumors are called cancer (Kumar V et al., 2006). About ten million people are diagnosed with cancer and six million dies every year all over the world due to cancer (Park K 2011). Chromosomal aberrations lead to tumor formation and cancer (Donna GA et al., 2003). The pioneering discovery by Muller (1927) on artificial mutagenesis in Drosophila opened a new horizon to the cytogenetic study of chromosomal aberrations. Sur PK et al., (1989, 2004, 2010) studied the effects of different doses of X-rays on male meiotic chromosomes of grasshoppers. Sur PK et al (2012 , 2015 a & 2015 b) also studied effects of different doses of X-rays & gamma rays on fish Oreochromis mossambicus ; mice Mus musculus and on shrew Suncus murinus . Kim HK et al., (2012) stated that cytotoxic chemotherapy remains the primary treatment option for many cancer patients. Search for a radio-protective agent had been intensely carried out by workers like Patt ,et al., (1949), Mozdarani and Nazari (2009), Jagetia et al.,(2003) etc. In traditional medicine, Barleria lupulina plant is externally used as an anti-inflammatory agent (Kanchanapoom T et al., 2001) and even against snake bites, dog bites, swelling due to fall, insect bite (Doss A et al., 2011), herpes simplex and herpes zoster (Wanikiat P et al., 2008). Even, anti-diabetic potential of this plant extract in rats had been reported (Suba V et al., 2004).

Sur PK (2012) has already reported & subsequently patented anti-clastogenic, anti tumor, anti-cancer and radiation protection activities of this plant extract on fish & mammalian models . In the present study, for the first time, potent anti clastogenic activity of Barleria lupulina Lindl in house musk shrew Suncus murinus has been reported .

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II. MATERIALS AND METHODS

2.1 Preparation of the plant extract

Barleria lupulina of Acanthaceae family (common name hophead Philippine violet) (Fig. (i)), was identified from Central National Herbarium, Botanical Survey of India, Howrah-711103, West Bengal, India (Ministry of Environment & Forests, Govt. of India). Leaves of this plant were cleaned, washed and dried in shade. Leaf extract was obtained from the dried leaves by running 20 cycles of Soxhlet Extraction (continuous hot extraction) using ethanol (99.9%). A part of this Barleria lupulina Ethanol Extract (BLEE) was used for TLC and phytochemical analysis.

Then the BLEE was dried to remove the ethanol, and dissolved in distilled water to make Barleria lupulina Plant Extract (BLPE). 1% of this stock solution was used for injection into shrew .

2.2 Experimental Protocol with animals

The animals were divided into following sets:

SET I (BLPE pre-treated Series): Shrew injected with BLPE at 1ml per 100 gm body weight, one hour before whole body γ -irradiation (1.2 Gy) from Cobalt (Co^{60})

SET II (CONTROL) Shrew exposed to whole body γ-irradiation (1.2 Gy) from Cobalt (Co^{60})

SET III (BLPE post-treated Series): Shrew injected with BLPE at 1ml/100 gm body weight, one hour after whole body γ -irradiation (1.2 Gy) from Cobalt (Co^{60})

Mitotic chromosomes from femur bone marrow cells of shrew (SET I, SET II, SET III) were studied for each set of animals after 1 hr, 16 hr and 48 hr of treatment.
The study is cleared by Animal Ethical Committee, Dept of Zoology, Kalyani University, West Bengal, India.

2.3 Preparation of metaphase plates and data scoring
The shrew were injected intraperitonially with 0.03% colchicine solution at 1 ml/ 100 gm body weight after the six different time intervals as mentioned above. After 1 hr of injection, the shrew were chloroformed and sacrificed. The femur bone was dissected out and bone marrow was suspended in 1 % sodium citrate solution and fixed in aceto-alcohol (acetic acid: alcohol – 1:3 v/v). Slide preparation was done by dropping fixed cells on clean grease free chilled slides (which were maintained at -5ºC), slides were heat fixed and air dried. Chromosomes were stained with Giemsa staining solution. Scoring of data was done by using 300 well-spread metaphase plates for each SET and each hour. Different types of structural chromosomal aberrations were studied.

2.4 Preparative Thin Layer Chromatography (TLC)
Preparative TLC was performed to identify active component in BLEE. For TLC, commercially available $60F_{254}$ (pore size; 60 Å, uv fluorescence at 254 nm, Merck, Germany) silica gel plates were used as the stationary phase whereas chloroform: ethyl acetate: ethanol (4: 3.5: 2.5) was used as the mobile phase. The mobile phase was chosen by trial-and-error method. A drop of BLEE was put on the silica gel plate and TLC was run using the mobile phase.

2.5 Phytochemical analysis
Phytochemical analysis was carried out for further identification of active component in BLEE. Standard phytochemical tests to study the presence of steroid, terpenoid, glycoside, flavonoid, alkaloid, tannin, saponin, carbohydrate, protein, fixed oil and pH were carried out by methods discussed by Trease GE and Evans WC (1997).

III. Results

3.1 Comparison between SET I, SET II and SET III shrew (Table (i))
300 cells for each hour for each SET were studied in these series (total 1800 cells and therefore 72000 chromosomes in each SET). Different types of structural chromosomal aberrations were studied (Table (i), Fig (ii)) and the study was done at various time intervals, viz: 1 hr, 16 hr, 48 hr (Table (i)). In case of SET II shrew, the frequency of chromosomal aberrations increased from 1 hour to 16 hours, was maximum at 16^{th} hour (10.25**%**) and then decreased thereafter. On the other hand, less frequency of aberrations was observed for SET I and SET III shrew. When compared with types of aberrations, much higher percentage of aberrations was observed with SET II shrew and much less aberrations had been scored with SET I animals and even lesser were scored with SET III animals (Table (i))

3.2 Statistical Analysis (Comparison between SET I and SET III shrew (Table (ii))
To evaluate the effectiveness of pre-treatment (SET I) and post–treatment (SET III) of BLPE on the shrew, statistical analysis was done according to methods mentioned by Snedecor GW (1967).
Analysis of the data reveals, that, in the pooled data, the Standard Error (SE), Critical Difference (CD) at 5%, CD at 1 % levels for SET I animals are 127.60, 250.10 and 308.57 respectively. And that for SET III are 87.92, 171.96 and 226.39 respectively. Therefore, the values shown by SET III are always less than the SET I. The t-Values, Chi-square values and r-Values are 7.57**, 51.58** and 0.90** respectively (significant at 1% level (highly significant)). Therefore, Post Treatment of BLPE (SET III) reflects significantly high protection than the Pre Treated series (SET I) (Table (ii)).Whereas, in SET II animals, chromosomal aberrations were much higher.
Therefore it may be inferred that BLPE provided protection against γ-ray (1.2 Gy) induced mitotic chromosomal aberrations in shrew to a significantly high level.

3.3 Preparative TLC
In preparative TLC, six spots (retention factor (R_f) values as 0.089, 0.196, 0.34, 0.46, 0.53, 0.84) were obtained (Fig. (iv)). No extra spot was obtained under uv (254 nm) fluorescence.

3.4 Phytochemical analysis
Phytochemical analysis of BLEE reveals the presence of steroid, terpenoid, glycoside, flavonoid, tannin and carbohydrate. This result corresponds to the appearance of six spots in TLC. The pH of BLEE had been found to be 5 (acidic).

Discussion

It was reported that three genes - MYC, EGFR and FGFR2 predicts poor survival in fluorouracil-treated metastatic gastric cancer patients (Kim HK et al., 2012). In previous studies, Mantena et al., (2008) reported radiation-induced aberrant metaphases and micro nucleated erythrocytes at 24 hr post exposure to 4 Gy γ radiations. Lemon et al., (2008) reported 2 Gy γ radiation DNA damage in whole body exposed normal and transgenic shrew. Even Jagetia et al., (2003) proved that naringin, a citrus flavonone, protects shrew from γ irradiated cellular DNA damage. Doss A et al., (2011) stated the antibacterial effects of extracts from Barleria lupulina and also evaluated its phyto-constituents.
In this study, it had been observed that in our shrew exposed only to γ-ray (1.2 Gy) (SET II), maximum percentage of aberration was obtained at 16^{th} hr (10.25%) (Table (i), Fig (iii). But on Pre-treating with BLPE, the percentage of aberration at 16^{th} hr is reduced to 5.28% (SET I) and in Post-treated series it was 3.16 % (SET III) (Table (i), (Fig (iii).
Therefore, it may be concluded that the extract from leaves of Barleria lupulina (BLPE) provided as an ameliorating agent against γ-ray induced structural chromosomal damage, in shrew, and post treatment of the plant extract had reduced chromosomal aberrations up to a significant level i.e. 54.83%, showing a great role in radiation-protection.
Repeated experiments of Sur PK et al (2012a & 2012b) on other doses of gamma-rays (0.8Gy, 1.2Gy and 2.4Gy) +

BLPE on other animal models as fish and mice also supported this view. Whole plant extract has already been patented in regard to its anti-clastogenic, anti tumor, anti-cancer and radiation protection activities.

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Table (i): Various chromosomal aberrations for shrew by gamma irradiation (1.2 Gy): SET I, SET II and SET III at different time intervals

Time interval	Animal SET	No. of cells	No. of chromosomes	Types of aberrations							Total Aberrations	Percentage of Total Aberrations
				chromatid break	iso chromatid gap	iso chromatid break	sub chromatid gap	centromeric dissociation	translocation	ring chromosome		
1 hour	SET I	300	12000	35	30	18	15	82	16	18	286	2.83 %
	SET II	300	12000	73	36	20	40	53	81	27	421	3.15 %
	SET III	300	12000	36	35	5	33	52	7	22	261	2.17 %
16 hour	SET I	300	12000	182	60	64	121	263	15	13	1068	5.28 %
	SET II	300	12000	257	143	155	170	186	28	80	1361	10.25 %
	SET III	300	12000	133	100	25	135	20	82	74	839	3.16 %
48 hour	SET I	300	12000	152	102	62	101	351	14	42	903	6.17 **%**
	SET II	300	12000	327	220	164	328	491	55	109	1996	9.86 %
	SET III	300	12000	86	4	2	301	80	10	20	554	4.67 %
Total Aberrations	SET I	1800	72000	945	365	232	578	857	225	239	4494	5.16 %
	SET II	1800	72000	1380	719	408	1053	1386	394	285	6635	6.82 %
	SET III	1800	72000	519	213	67	686	365	155	181	2916	3.08 %
Percentage of Total Aberrations			SET I	1.31 %	0.51 %	0.32 %	0.80 %	1.19 %	0.31 %	0.33 %		
			SET II	1.92 %	1.00 %	0.57 %	1.46 %	1.93 %	0.55 %	0.40 %		
			SET III	0.72 %	0.30 %	0.09 %	0.95 %	0.51 %	0.22 %	0.25 %		

Table (ii): Statistical analysis to compare between SET I and SET III shrew (gamma ray 1.2 Gy)

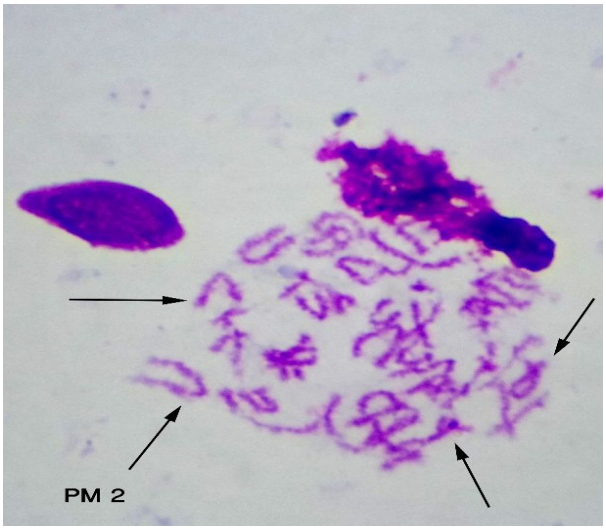
Aberration Statistics	NC with aberration	Chromatid break	Isochromatid gap	Isochromatid break	Subchromatid gap	Centromeric dissociation	Translocation	Ring Chromosome	Rabbitear chromosome	Rabbitear chromosome with gap	Pooled
SET I +/- S.E.	**11.06**	**37.71**	**18.45**	**8.79**	**21.33**	**53.41**	**14.26**	**11.23**	**25.79**	**28.27**	**127.60**
SET III +/- S.E.	**27.87**	**18.85**	**14.97**	**4.40**	**41.73**	**15.16**	**11.67**	**8.97**	**28.57**	**8.91**	**87.92**
SET I C.D. at 5%	**21.68**	**73.91**	**36.16**	**17.22**	**41.82**	**104.69**	**27.95**	**22.01**	**50.54**	**55.40**	**250.10**
SET III C.D. at 5%	**54.51**	**36.87**	**29.29**	**8.61**	**81.62**	**29.65**	**22.83**	**17.54**	**55.88**	**17.43**	**171.96**
SET I C.D. at 1%	**28.48**	**97.10**	**47.50**	**22.62**	**54.94**	**137.54**	**36.72**	**28.92**	**66.40**	**72.79**	**308.57**
SET III C.D. at 1%	**71.76**	**48.54**	**38.55**	**11.33**	**107.45**	**39.04**	**30.05**	**23.09**	**73.56**	**22.95**	**226.39**
t - Values	**12.12****	**6.27****	**2.57**	**5.98****	**1.18**	**3.70***	**1.63**	**1.52**	**4.31****	**1.57**	**7.57****
Chi-Square (χ^2)Values	**68.72****	**29.96****	**197.20****	**58.65****	**168.52****	**259.34****	**111.27****	**101.52****	**92.66****	**96.45****	**51.58****
r - Values	**0.08**	**0.84****	**-0.24**	**-0.07**	**0.25**	**-0.10**	**-0.12**	**-0.48**	**0.64****	**0.64****	**0.90****

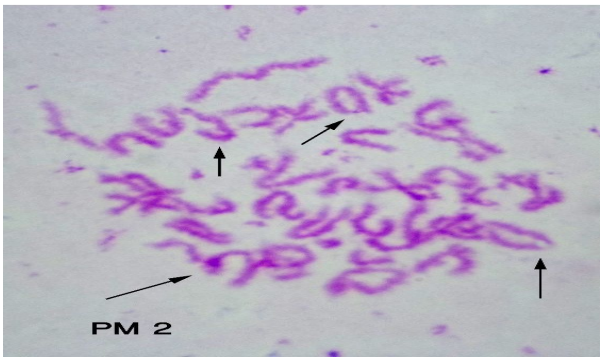
* Significant at 5% level
** Significant at 1% level

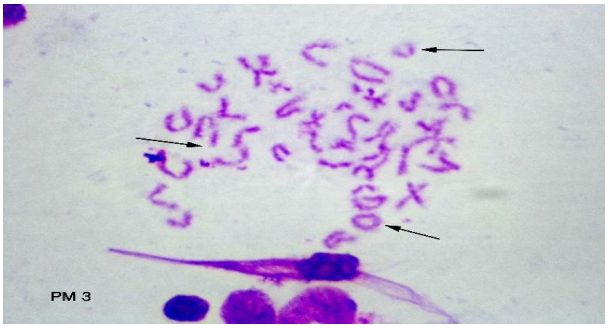
Statistical analysis of the data were undertaken as Standard Error (S.E) ; Critical Difference (C.D) at 1% and 5 % level; t- test ; chi- square(χ^2) analysis and correlation coefficient (r-value) of both the series. NC= Number of cells

Fig (i): Shrew after dissection

Fig.(ii): Barleria lupulina plant with flower pod







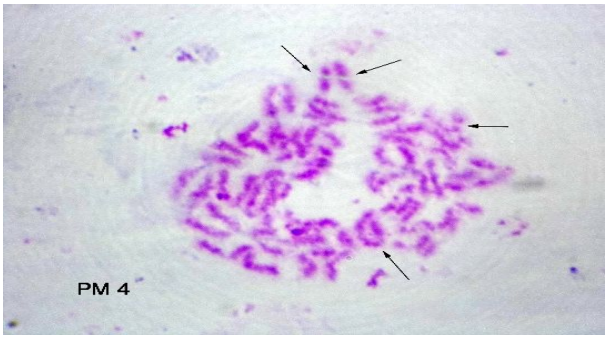


Fig (iii): Photomicrographs (PM) showing various types of structural chromosomal aberrations in shrew Post-Treated with BLPE (SET III)

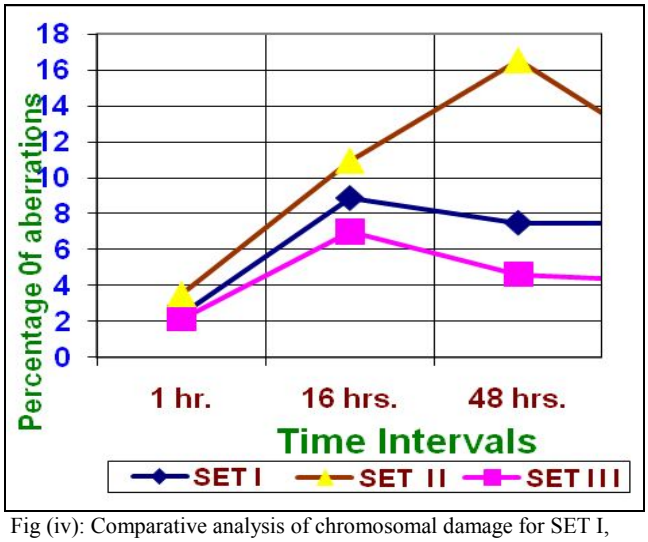


Fig (iv): Comparative analysis of chromosomal damage for SET I, SET II and SET III shrew with respect to 3 different time intervals