



Studies on anti-genotoxic effect of *Piper betle* leaves

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Abstract

Objective: To study the antigenotoxic effect of *Piper betle* leaves in Gamma irradiation and Cyclophosphamide treated animals. **Materials and methods:** Swiss albino mice were given 50% methanolic extract (100mg/kg body weight.) of *Piper betle* leaves and antigenotoxic effect was studied after whole body gamma irradiation (4Gy) & cyclophosphamide (50mg/kg body weight.) treatment. Bone marrow protection was studied by scoring aberration in metaphase chromosomes. **Results:** No drug toxicity was observed at given dose (100mg/kg body weight.) Methanol extract ½ hr prior to irradiation protects animals against Gamma irradiation & Cyclophosphamide treatment.

Key words: Anti-genotoxic activity, *Piper betle*, Gamma irradiation, Cyclophosphamide.

1. Introduction

Exposure of mammalian systems to radiations and cyclophosphamide induces damaging effects leading to cell death and an increased risk of diseases particularly cancer [1]. A dose of 4 Gy is considered fatal for humans and other mammals [2]. Consequently, there is growing interest in developing new radioprotectants in preventive medicine as well as adjuvant therapy. Most of the effective radioprotectants such as WR-2721 developed so far are synthetic, and are reported to be toxic [3]. Thus, there is a need to develop radioprotectants from natural sources especially from edible or medicinal plants/herbs as these are regarded as non-toxic

even at higher concentrations. The importance of usage of ethnomedicines is increasing nowadays as they have less or no side effects, low cost and are, often easily accessible to the common people. Almost half of the pharmaceuticals are originated from plant products. The present study was primarily aimed to this end. Wherein the radioprotecting property of *Piper betle* Linn. Commonly known as tambula (Sanskrit), pan (Hindi and Bengali) of piperaceae family was studied.

The *Piper betle* plant is widely growing in the tropical humid climate of South East Asia and its leaves, with a strong pungent and aromatic flavor

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are widely consumed as a mouth freshener. The leaves are credited with wound healing, digestive and pancreatic lipase stimulant activities in the traditional medicine [4, 5], which has also been proved with experimental animals [6].

In fact, usefulness of this plant against various diseases can be traced in the ancient Vedic literature, *Atharved* as early as 3000-2500 BC. Earlier, we also reported gastrocytoprotective properties of the leaf extract on experimentally induced gastric lesions and rationalized the activity in terms of its antioxidant property [7, 8]. In addition, its antimicrobial [9], antifungal and anti-inflammatory [10] activities are also reported.

2. Materials & Methods

Animals - Swiss albino mice of either sex weighing 30-40gm were obtained from Jawaharlal Nehru Cancer Hospital and Research Center. Animals were kept in cages and in environmentally controlled room. ($23 \pm 3^{\circ}\text{C}$, 12hr light & dark cycle) with free access to water. Animals were fed with standard diet pellets *ad libitum*. Animals randomly allocated to different experimental groups, three or four mice were used for each groups.

Experimental protocols were approved by institutional ethical committee of JNCH & RC, Bhopal, which follow guidelines of CPCSEA (Committee for the Purpose of Control & Supervision of Experiments on Animals) that complies with international norms of INSA.

2.1 Collection of Plants

The fresh leaves of *Piper betle* were collected from the region of Madhya Pradesh (Bhopal) in the month of February and were identified by Botanist Professor Shaukat Ali, Safia College, Bhopal.

Fresh leaves were washed under tap water and shade dried and powdered. 50% methanolic

extract of the powder (100gm) was prepared with the help of cold maceration, at room temperature for about 18 hrs, shaking frequently. The process of extraction was repeated for three times. The combined extracts were filtered and concentrated under vacuum using SC 110A Speed Vac plus at 4°C . the extractive value of extract obtained was 14.94% w/w on dry basis.

Treatment schedule – Animals were divided into following seven groups of three to four animals each.

Group – I (control) – Double distilled water (DDW)

Group – II (Vehicle alone) - Iso propyl alcohol (IPA)

Group – III (Methanolic extract) - 50% Methanolic extract of *Piper betle* (dose 100mg/kg)

Group – IV Radiation alone (4 Gy)

Group – V Radiation plus methanolic extract of *Piper betle* (dose 100mg/kg)

Group – VI Cyclophosphamide alone (50mg/kg) (CP)

Group – VII Cyclophosphamide plus methanolic extract of *Piper betle* (dose 100mg/kg)

All the above-mentioned groups were treated i.p. Methanolic extract dissolved in IPA.

2.2 Mode of treatment

Irradiation : The cobalt therapy unit (Canada) in the Jawaharlal Nehru Cancer Hospital, Bhopal was used for irradiation. Unanaesthetized animals were restrained in well ventilated Perspex boxes and whole body exposed at a dose rate of 4Gy/minute at a SSD of 112cm. [11].

Chemotherapy : An intraperitoneal (i.p.) injection of 50mg/kg Cyclophosphamide i.p (Dabar, New Delhi) was administered alone as well as with extract.

Table 1. Different types of chromosomal aberration in mice bone marrow cells 24 hr after irradiation & chemotherapy

Treatment	Aberrant cells/500 metaphase	Aberrations/cell				Ring	Fragments	Total aberrations
		Chromosome breaks	Chromatid breaks	Dicentric				
Control	2.33 ± 0.33	0.066±0.066	0.2 ± 0.115	0.00		0.00	0.466±0.066	0.733±0.133
Vehicle alone IPA	2.66 ± 0.88	0.066±0.066	0.266±0.066	0.00		0.00	0.533±0.176	0.866±0.240
Methanolic extract alone (<i>P.belle</i>)	4.0 ± 0.577	0.533±0.066	0.466±0.066	0.00		0.066±0.066	1.133 ± 0.066	2.2 ± 0.0115
Radiation alone (4 Gy)	190.0 ± 4.41 ^{c,f}	7.45 ± 0.65 ^{b,e}	8.4±0.454 ^{b,e}	1.85 ± 0.15		2.25±0.125 ^{a,e}	28.75±0.713 ^{c,f}	48.7±1.340 ^{c,f}
Radiation + Methanolic extract (<i>P.belle</i>)	123.5 ± 2.986 ^{c2,f}	4.5±1.29 ^{b,1,e}	4.7±0.057 ^{c,1,f}	1.35±0.095 ^{a,d}		2.15 ± 0.170 ^e	18.8±0.707 ^{b,1,e}	31.5±0.613 ^{c1,f2}
Cyclophosphamide alone	235.75 ± 4.385 ^{c,f}	9.4±0.454 ^{b,d}	9.7±0.264 ^{c,d}	3.05±0.15 ^{a,d}		2.5 ± 0.057 ^{a,e}	31.05±0.623 ^{c,e}	55.7±1.096 ^{c,f}
Cyclophosphamide + Methanolic extract (<i>P.belle</i>)	171.25 ± 3.326 ^{c,f}	6.5 ± 0.129 ^{c,x,f}	7.1±0.129 ^{c,x,f}	3.5±0.129 ^{a,d}		4.35±0.15 ^{a,y,e}	19.65±0.665 ^{b,y,e}	41.1±0.544 ^{c1,y}

a, 1, x, d = p < 0.05; b, 2, y, e = p < 0.01 c, 3, z, f = p < 0.001

a, b, c is compared to DDW (control); 1, 2, 3, is compared to RT alone; x, y, z is compared to CP alone; d, e, f is compared to ME alone.

Table 2. Different types of aberrant metaphases in the bone marrow of mice on one day after treatment with *Piper belle* methanolic extract, Radiation (RT 4Gy) and Cyclophosphamide (50 mg/kg)

Treatment	Total Aberrant Metaphase	TYPES OF ABERRATION (PERCENT)			SDC
		Polyploidy	Pulverization		
Control	0.00	0.00	0.00		0.00
Vehicle alone IPA	0.00	0.00	0.00		0.00
Methanolic extract alone (<i>P. belle</i>)	0.00	0.00	0.00		0.00
Radiation alone (4 Gy)	10.6 ± 0.24 ^{a,d}	1.75 ± 0.170	4.55 ± 0.15 ^{a,d}		4.3 ± 0.129 ^{a,d}
Radiation + Methanolic extract (<i>P. belle</i>)	5.6 ± 0.081 ^{b,e,2}	1.6 ± 0.081 ^{a,d}	1.45 ± 0.095 ^{a,2,d}		2.55 ± 0.095 ^{a,2,d}
Cyclophosphamide alone	20.8 ± 0.29 ^{b,e}	5.55 ± 0.05 ^{b,e}	7.75 ± 0.170 ^{a,d}		7.5 ± 0.129 ^{a,d}
Cyclophosphamide + Methanolic extract (<i>P. belle</i>)	11.7 ± 0.28 ^{a,d,y}	3.6 ± 0.115 ^{a,y,d}	3.5 ± 0.129 ^{a,y,d}		4.6 ± 0.115 ^{a,y,d}

a, 1, x, d = p < 0.05; b, 2, y, e = p < 0.01 c, 3, z, f = p < 0.001

a, b, c is compared to DDW (control); 1, 2, 3, is compared to RT alone; x, y, z is compared to CP alone; d, e, f is compared to ME alone.

Chromosomal assay : The mice were injected i.p. with 0.025% colchicine (Sigma, USA) and left for 2 hrs, to arrest the cell in metaphase. Then the animals were sacrificed by cervical dislocation, femur were dissected out and cleaned to remove adherent muscles. Metaphase spreads were prepared by the air-drying method [10]. Briefly, the bone marrow cells were flushed out, treated with hypotonic saline, fixed in methanol : acetic acid, stained with 1% Giemsa, and observed under a light microscope (Leica 80,2000). A total of 500 metaphase spreads were scored per animal and the number of aberrant cells were counted and expressed as percentage of total metaphases scored. The individual aberration namely chromosomes and chromatid breaks, fragments, rings and dicentric as well as cells with polyploidy, pulverization and severely damaged cells. (SDC, i.e. cells containing 10 or more aberrations were also scored).

2.3 Statistical Analysis

The data were analyzed by student's test. Comparisons between different groups were done by one-way ANOVA (kypilot) using Graph PAD instant software (USA).

3. Results

The control group (DDW) showed $0.73 \pm 0.133\%$ of aberrant cells. The different types of aberrations like fragments, chromatid and chromosome breaks were found. The animals treated with vehicle alone (IPA) did not show much toxicity and the aberration found were not significantly different when compared to the same treated controlled group (Table 1,2.).

The animals with methanolic extract of *Piper betle* (100mg/kg b.w.) was administered i.p. were scored with less aberration not significant when compared with control (DDW). Radiation (4Gy) produced a significant increase in the percent aberrant metaphases and different types

of aberrations compared to same treated control. Radiation increased all types of aberrations like fragment chromatid and chromosome breaks, rings and dicentric.

Quantitatively, methanolic extract pretreated group showed similar aberrations as in the RT alone group. But treatment with methanolic extract of *Piper betle* before radiation significantly ($p = < 0.05 - 0.001$) reduced percentage aberrant metaphases and different types of aberrations (Table 1,2.).

There was a significant reduction in case of pulverized cells when compared to radiation alone group.

CP (50mg/kg) increased the aberrant metaphases and chromosomal aberrations when compared to control vehicle alone and methanolic extract alone groups. CP produced $55\% \pm 1.096\%$ of total aberrations and also increases the unstable aberrations ($P = < 0.05 - 0.001$). The number of aberrant metaphases induced by CP was more than 1.5 times, to that produced by 4Gy of Gamma radiation.

Pretreatment with methanolic extract of *Piper betle* resulted in $\leq 12\%$ reduction in chromosomal aberrations when compared to CP alone group (Table 1,2).

4. Discussion

This study demonstrates a radioprotective as well as chemoprotective property of the *Piper betle* leaf extract. The data clearly show that a signal dose of 100 mg/kg methanolic extract of *Piper betle* can significantly decrease the chromosomal damage caused by Gamma irradiation & Cyclophosphamide. Administration of the methanolic extract further enhanced the bone marrow protection, as indicated by the significant reduction in the chromosomal aberrations at 24 hrs, after irradiation and CP (administered intraperitoneally).

The radioprotective and chemoprotective effect of several natural products has been associated with their antioxidant property. Earlier studies from other laboratories have been shown that *Piper betle* possesses antioxidant activities.

5. Conclusion

Thus, the present study demonstrates that nontoxic doses of an extract of *Piper betle* leaves protect bone marrow chromosomes exposed to whole body Gamma irradiation and

Cyclophosphamide. *Piper betle* leaves have been reported to contain the antioxidants like vitamin C, along with other constituents, which may be responsible for the radioprotective and chemoprotective properties of the extract. *Piper betle* leaves freely available in India, are used as flavors to the preparation and as a spice in different curries. It is worthwhile to conduct detailed studies in order to explore the full potential of this plant in human radioprotection and chemotherapeutic protection.

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