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Role of *Pistia stratiotes* on Radiation Induced Genotoxicity : Analysis of Micronucleus and Chromosome Aberrations *in vivo*

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Abstract **-** The present study aimed to examine the radioprotective potentiality and efficacy of *Pistia stratiotes* against damage induced by ^{60}Co gamma radiation. Mice were exposed to 4 Gy ^{60}Co gamma rays. MeOH extract (50 and 100 mg/kg body wt) was administered orally with irradiation. Bone marrow protection was evaluated by scoring the different types of individual aberrations, aberrant metaphases and micronuclei formation. Significant reduction in number of aberrant cells and different type of aberrations was observed in treated group compared to irradiated untreated group of animals. The administration of MeOH leaf extract of *Pistia stratiotes* to the animals showed significant reduction in micronucleus induction. The presence of large amount of two di-Cglycosylflavones of the vicenin and lucenin and lesser amounts of the anthocyanin cyaniding-3-glucoside and a luteolin-7-glycoside, and traces of the mono- C-glycosyl flavones, vitexin and orientin found to be responsible for the observed bioefficacy. Our findings support the use of *Pistia stratiotes* (ignored weed) as health promoting food and its potential for clinical use.

Keywords *- Pistia stratiotes, vitexin, orientin.*

I. INTRODUCTION

High levels of gamma irradiation can induce mortality in mammals. With respect to radiation damage to humans, it is important to protect biological systems from radiation induced genotoxicity or lethality. The main radioprotective class is thiol synthetic compounds such as amifostine. Amifostine is a powerful radioprotective agent compared with other agents, but this drug is limited in the use in clinical practice due to side effects and toxicity [1-3]. Attention has been shifted towards the evaluation of plant products as radioprotectors, in the last 15 years, due to their efficacy and low toxicity. The proposed radioprotective efficacy of plant extracts is a result of their containing a large number of active constituents, such as antioxidants, immunostimulants, and compounds with antimicrobial activity. Therefore, screening herbal drugs offers a major focus for new drug discovery[4]. Various drugs from natural or synthetic origin, i.e., antioxidant cytoprotective agents, immuno modulators and DNA binding molecules, have been evaluated extensively for their radioprotective potentials in both *in vitro* and *in vivo* models [5,6,7,8,9]. However, the fact remains that there is not a single radioprotective drug available which meets all the prerequisites of an ideal radioprotector. In view of this, the search for less toxic and more potent radioprotector drugs continues.

Several research studies have demonstrated that herbal plants contain diverse classes of compounds such as steroids, polyphenols, alkaloids, tannins and carotenoids[10]. Many flavonoids and lignans are already known for their antioxidant action and anti-apoptotic potential, and thus contribute towards radioprotection [11]. From the previous research it was found that *Pistia stratiotes* L. contains large amount of two di-Cglycosylflavones of the vicenin and lucenin and lesser amounts of the anthocyanin cyaniding-3-glucoside and a luteolin-7-glycoside, and traces of the mono- C-glycosyl flavones, vitexin and orientin[12]. With this background and presence of abundant source of unique active components in *Pistia stratiotes*, the present study was taken up on this plant namely *Pistia stratiotes* belongs to the family Araceae. *Pistia stratiotes* is used in traditional medicine for its diuretic, antidiabetic, antidermetaphytic, antifungal and antimicrobial properties [13].

Recently, we reported that *P. stratiotes* extract possess *invitro* antioxidant [14] and anticancer activity [15] against melanoma cell lines. In continuation of this line of investigation, the *in vivo* radioprotective activity of *P. stratiotes* was assessed by using gamma rays as an oxidative DNA damaging agent and in evaluating any reduction in chromosomal aberrations and micronucleus formation in mouse bone marrow cells exposed to gamma irradiation.

II. MATERIALS AND METHODS

A. Authentication of Plant

The *Pistia stratiotes* leaves were collected from upper lake, Bhopal (M.P), India during the month of October. Further taxanomic identification was conducted by taxonomist, Department of Botany, Safia College of Science, Bhopal (M.P.), India. A voucher specimen (296/Botany/Safia/11) has been deposited to the Botany Department.

B. Plant material and preparation of leaf extract

The collected plant material was dried under shade and then powdered with mechanical grinder. The powder (200 g) of *P. stratiotes* was extracted with maceration at 40°C with 4 L mixture of methanol: distilled water (50/50, v/v). The macerated mixture was filtered through muslin cloth and the solvent was removed under reduced pressure (p = 120 mm Hg) to yield 15 g of dark solid extract. The extract was stored at a temperature of 4ºC for further investigations.

C. Determination of total phenolic content [16]

1.0 ml of extract solution containing 1.0 ml extract in a volumetric flask was diluted with 46 ml of distilled water. 1.0 ml of Folin-Ciocalteau reagent was added and mixed thoroughly. Three minutes later, 3.0 ml of 2% sodium carbon- ate was added and the mixture was allowed to stand for 3 h with intermittent shaking. The absorbance of the blue color that developed was measured at 760 nm. The concentration of total phenolic content was expressed as mg/g of dry extract.

D. Determination of flavonoids content [17]

Briefly, 1 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract. Absorption readings at 415 nm were taken after 10 min against a blank sample consisting of a 1 ml extract solution with 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the equation that was obtained from the standard rutin graph.

E. Animals

Swiss albino mice weighing 25±5 gm were maintained in ventilated animal house of Department of Research, JNCH & RC, Bhopal (India). All mice were kept at controlled environmental condition (22±2 °C, 60±5 % humidity) with 12 hrs light/ dark cycle. Necessary approvals were obtained from the IAEC, Dr. Hari Singh Gour University, Sagar (M.P) India, (CPCSEA Registration no –379/01/ab/CPCSEA/2010

F. Irradiation

Mice were irradiated by 60Co source in the cobalt teletherapy unit (Siemens, Munich, Germany) at the Radiation Oncology Department, JNCH & RC, Bhopal (India) with an isocentric three-beam technique. The source to skin distance (SSD) was 80 cm with irradiation time 1.89 min. Radiation was given simultaneously, 4 Gy in two fractions at 9^{th} and 12^{th} day of treatment schedule.

G. Experimental dose

Mice were randomized and divided into five groups of six animals each:

Group 1: Control (Normal saline only),

Group 2: *P. stratiotes* only (100mg/kg bw),

Group 3: Radiation control group (receives 4Gy radiation in two fraction on day 9^{th} and 12^{th}),

Group 4: Treatment group (along with radiation mice were given *P. stratiotes* 50mg/kg bw) orally.

Group 5: Treatment group (along with radiation mice were given *P. stratiotes* 100mg/kg bw) orally.

H. Metaphase Preparation

After 24 hr of treatment protocol of 20 days all the animals were injected i.p. with 0.025% colchicine and sacrificed 2h later to arrest the cells at metaphase by cervical dislocation. Both femurs were dissected and cleaned to remove the adherent muscles and metaphase plates were prepared by air-drying method [18]. The bone marrow cells were flushed out, treated with pre-warmed 57 % hypotonic saline, fixed in carnoy's fixative, stained with 4 % Giemsa and observed under a light microscope. A total of 400 metaphase spreads were scored per animal and the number of aberrations, namely chromosome and chromatid breaks, fragments and rings were scored.

I. Micronucleus assay

The *micronucleus* assay is a biological dosimeter of *in vivo* ionising radiation exposure. The bone marrow was flushed out using minimum essential medium and the slides were prepared by the method of Schmid, 1975 [19]. Bone marrow was centrifuged and the pellet was resuspended in few drops of fetal bovine serum. Smears were prepared on preclean glass slides, stained with May-Grawnwald and followed by Giemsa stain and observed under a light microscope for micronuclei in polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs).

J. Statistical analysis

All experimental data are given as mean ± SD. Statistical analysis was carried out using the one-way analysis of variances (ANOVA) using Graph Pad Prism software. Probability values were found to be equal to or less than 0.05.

III. RESULTS AND DISCUSSION

A. Total phenolic content :

Standard curve of gallic acid (1µg/ml to 150µg/ml) have the regression coefficient value was 0.961 with line regression y = 0.001x + 0.037 and the total amount of phenol present in the *P. stratiotes* is shown in Fig. 1. In 100 gram of *P. stratiotes* extract, 54.5±0.05 mg gallic acid (Table 1) equivalent of phenols was detected.

B. Determination of flavonoids content:

Rutin is a flavonol glycoside plant metabolite reduces the fragility of blood vessels found in haemorrhagic disease and hypertension in humans [20].

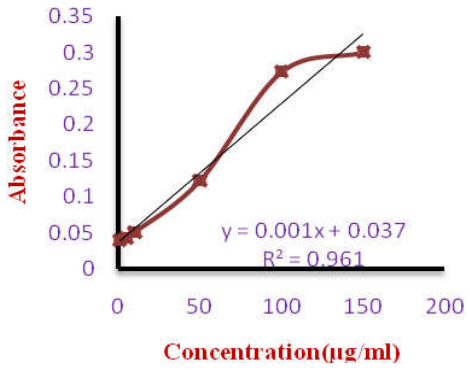

Fig.1 : Standard Curve for Gallic acid

In this study, rutin was used as standard. The *P. stratiotes* extract contained 75.3±0.08 mg of rutin equivalents flavonoids (Fig 2). The total flavonoid content of *P. stratiotes* extract was expressed as rutin equivalents in mg/g of extracts.

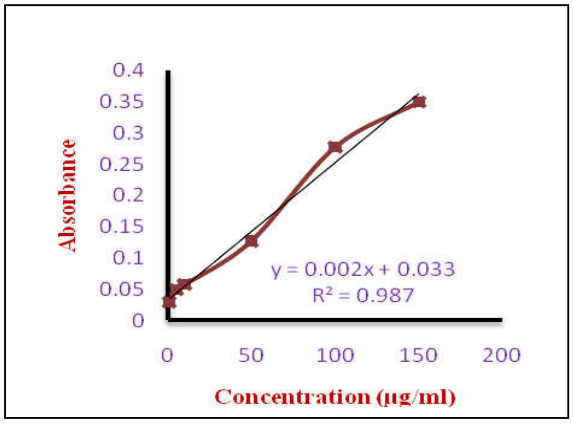

Fig. 2 : Standard Curve for Rutin

Table 1: Amount of Total phenolics (mg gallic acid equivalent/100mg) and flavonoids (mg rutin equivalent/100mg)

Extract	Total phenols	Flavonoids
P. stratiotes	54.5±0.05 mg	75.3±0.08 mg

C. Effect of P. stratiotes on radiation-induced Chromosomal aberrations

Chromosome aberrations have been used as a sensitive monitor of DNA damage in studies of several radioprotectors. In the study, Control group showed less than 2% aberrant cells, treatment of extract alone did not induce any significant changes compared to control. Radiation produced a significant increase in the percent aberrant cells. A corresponding increase was found in all the individual aberrations. Treatment with MeOH extract of *P. stratiotes* leaves and irradiation resulted in very significant decrease in the percent chromosomal aberration like chromatid breaks, centric rings, acentric fragments, pulverization and total abnormal metaphases in bone marrow cells compared to normal control (Table 2).

D. Effect of P. stratiotes on radiation-induced micronuclei formation

Whole body radiation resulted in significant increase in MN in PCE (215/1510) and MN in NCE (8/2306) and a significant reduction in the P/N ratio (0.65) as shown in Fig.3. Treatment with *P. stratiotes* reduced micronucleus formation 6/1505 MN in PCE and 1/1499 MN at the concentration of 100 mg/kg body wt. The P/N ratio increased from 0.65 in the irradiated group to 0.79 (50 mg/kg body wt) and 0.80 (100 mg/kg body wt) by administration of the extract.

Table 2 : Effect of P. stratiotes extract on the induction of chromosomal aberrations in mouse bone marrow by whole body gamma-irradiation (4Gy).

Treatment groups	Types of chromosomal aberrations Mean ± SD						
	Fragments	Deletion	Ring	Break	Double Minute	Pulverization	% Abberation
Control	1.44 ± 1.15	1.83 ± 0.44	-	-	2.2 ± 0.43	-	1.61 ± 0.38
Extract only (100mg/kg bw)	-	-	-	1.20 ± 0.16	-	-	0.02 ± 0.01
Radiation control (4Gy)	20.94 ± 0.87	21.55 ± 1.46	6.77 ± 1.72	5.38 ± 1.80	15.1 ±1.27	23.5 ± 2.21	20.94 ± 2.16
Radiation 4 Gy+ MeOH Extract (50mg/kg)bw	10.72 ±1.00*	11.61 ± 2.25*	5.20 ±1.46*	4.11±1.98*	13.0±1.62*	16.11±1.62*	17.83± 1.90*
Radiation 4 Gy+ MeOH Extract (100mg/kg)bw	8.83 ± 0.77 *	8.55 ± 1.73*	2.05±0.87*	3.22±1.73*	10.8±1.04*	12.38±0.87*	13.61± 0.22*

Mean ± SD (n=6). P value: P>0.05 vs. Radiation Control

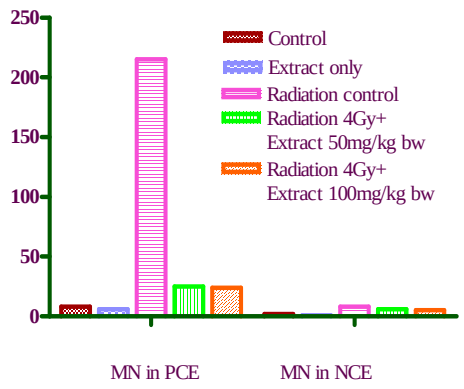

Fig. 3 : Effect of the *P. stratiotes* on frequency of micronuclei.

IV. CONCLUSION

The protective effect of *P. stratiotes* extract was seen against the mutation induced by radiation. Damage to the chromosomes is manifested as breaks and fragments which appear as micro-nuclei in the rapidly proliferating cells [21]. The tests were performed on mice bone marrow for chromosomal aberration assay and micronucleus assay. All the results find statistically significant. MeOH extract of *P. stratiotes* was notably capable of significant reduction of chromosomal aberration and micronuclei formation Therefore, from the present study, it can be concluded that *P. stratiotes* possesses radioprotective potential.

REFERENCES

[1] SJ. Hosseinimehr, A Shafiee, H Mozdarani, S Akhlagpour, "Radioprotective effects of 2-iminothiazolidine derivatives against lethal dose of gamma radiation in mice," J Radiat Res.42, 2001, 401–8.

[2] SJ Hosseinimehr, A Shafiee, H Mozdarani, S Akhlagpour, M Froughizadeh, "Radioprotective effects of 2-imino- 3{(chromone-2-yl) carbonyl} thiazolidine against gamma irradiation in mice," J Radiat Res. 43, 2002, 293–300.

[3] AT Turrisi, DG Glover, S Hurwitz, "The final reports of the phase I trial of single dose WR-2721, s-2-(3-aminoprpylamino) ethyl phosphorothioic acid," Cancer Treat Rep. 70, 1986, 1389–93.

[4] SJ Hosseinimehr, "Trends in the development of radioprotective agents," Drug Discovery Today. 12, no. 19-20, 2007, 794–805.

[5] R Arora, D Gupta, R Chawla, R Sagar, A Sharma, R Kumar, J Prasad, S Singh, N Samanta and RK Sharma, "Radioprotection by plant products: Present status and future prospects," Phytotherapy Research. 19, 2005, 1-22.

[6] M Bala and HC Goel, "Radioprotective effect of podophyllotoxin in *Saccharomyces cerevisiae*," J of Environmental Pathology Toxicology and Oncology. 23, 2004, 139-144.

[7] A Cairnie, "Adverse effects of WR-2721." Radiation Research. 94, 1983, 221-226.

[8] WF Dorr, R Noack, R Spekl, K Farell, "Modification of oral mucositis by keratinocyte growth factor: single radiation exposure," International J of Radiation Research 77, 2001, 341-347.

[9] MM Kligerman, DJ Gloverand AT Turrisi, "Toxicity of WR-2721 administered in single and multiple doses," International J of Radiation Oncology Biology Physics. 10, 1984, 1773-1776.

[10] P Nandi, G Talukdar, A Sharma, "Chemopreventive agents: Activity and their mechanism," Pharmacotherapy.41, 1998, 53-5.

[11] R Chawla , R Arora, RK Sagar, S Singh, SC Puri, R Kumar, S Singh, AK Sharma, J Prasad, HA Khan, RK Sharma, KL Dhar, M Spiteller and GN Qazi, "3-O-beta-D-Galactopyranoside of quercetin as an active principle from high altitude *Podophyllum hexandrum* and evaluation of its radioprotective properties," J of Biosciences. 60, 2005, 728-38.

[12] Thomas M. Zennie and Jerry W. Mc Clure, "The falvanoid chemistry of *Pitia stratiotes* L. and the origin of the Lemnaceae," Aq. Botany. 3, 1977, 49-54.

[13] KR Kirtikar, BD Basu, 'Indian medicinal plants," volume IV, 2nd ed. Dehradun, Lalit Mohan Basu. 1994, 2600-2602.

[14] M Jha, N Ganesh, V Sharma, "*In vitro* evaluation of fee radical scavenging activity of *Pistia stratiotes*," International J of Chemtech Research. 2(1), 2010, 180-84.

[15] M Jha, V Sharma, N Ganesh, "*In vitro* Antioxidant and Cytotoxicity assay of PSLE against B16F1 and B16F10 melanoma cell lines," Springer e-book, Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives, Springer-Verlag Berlin Heidelberg, 4, 2011,19-23.

[16] K Slinkard, VL Singleton, "Total phenol analyses: automation and comparison with manual methods," Am J Enol Viticult. 28, 1977, 49–55.

[17] A Arvouet-Grand, B Vennat, A Pourrat, P Legret, "Standardisation d'un extrait de propolis et identification des principaux constituants," Journal de Pharmacie de Belgique. 49, 1994, 462–468.

[18] P Uma Devi, KS Bisht and M Vinita, "A comparitive study of radioprotection by ocimum flavanoids and synthetic aminothiol protectors in the mouse,' Br J Radiol. 71, 1998, 782-784.

[19] W Schmid, "The micronucleus test," Mutat Res. 31, 1975, 9-15.

[20] BD Oomah, CG Campbell, G Mazza, "Effects of cultivar and environment on phenolic acids in buckwheat," Euphytica .90,1, 1996, 73–77.

[21] M Hofer, L Mazur, M Pospisil and V Znogil, "Radio protective action of extracellular adenosine on bone marrow cells in mice exposed to gamma rays assayed by the micronucleus test," Radiat, Res.154, 2000, 217-221.

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