(IJPPS) 2017, Vol. No. 1, Issue No. I, Oct-Dec **ISSN: 2457-0419**

# PROTECTIVE ROLE OF PODOPHYLLUM HEXANDRUM IN RADIATION INDUCED HEMATOPOIETIC INJURY[1]

**Dr. Geeta Yadav**

*Received: 30 July 2017; Accepted: 30 September 2017; Published: 14 October 2017*

## INTRODUCTION

Hematopoietic system plays very important role in normal function and survival of organisms. A number of reports have validated deleterious effect of radiation on hematopoietic system. The hematopoietic system provides mature functional circulating elements since these have finite life times and cannot reproduce themselves, those lost through the bone marrow does not function if the length of time in which the hematopoietic system does not function too long, the level of vital circulating elements drops too low and the irradiated organism dies. The bone marrow tissues have very rapid turnover times that means constant source of new cells are supplied in order to balance the numbers that are lost.

## *PODOPHYLLUM HEXANDRUM*

*P. hexandrum* is an erect, glabrous, fleshy or succulent rhizomatous herb and is found growing in Himalayan region.it belongs to the family Berberidaceae/Podophyllaceae. The roots and rhizome of the plant have been used in the Indian as well as traditional Chinese system of Medicine for the treatment of diseases. The fruit of *Podophyllum hexandrum* is ate as a mild laxative and the rhizome is consumed as a purgative, hepatic stimulant and is applied intravaginally to treat gynecological infection since ages. Podophyllotoxin, a major component of *Podophyllum* has several medicinal application e.g., as an anti-malarial, anti-fungal, and possesses immunomodulatory activity.

## MATERIALS AND METHODS

### Animals

Swiss albino strain „A‟ male mice were used in these studies. The age of these mice were 12-15 weeks. These animals were kept in animal house which are maintained at 21˚C ± 2˚C with 50% ± 10% humidity on a 12-hr light/dark cycle. The animals were fed ad libitum with rodent food pellets (Amrut Laboratory

[1] *How to cite the article:* Yadav G.. Protective Role of Podophyllum Hexandrum in Radiation Induced Hematopoietic Injury, *IJPPS*, Oct-Dec 2017, Vol 1, Issue 1, 9-15

(IJPPS) 2017, Vol. No. 1, Issue No. I, Oct-Dec **ISSN: 2457-0419**

feed, New Delhi, India) and had free access of water.

Swiss Albino Strain „A" Mouse

**Preparation of drug compound**

*P. hexandrum* extract was dissolved in DMSO (Dimethyl sulphoxide) on the day of experiment and administered intramuscularly before 1 hr of irradiation.

**Radiation procedure**

Mice were received lethal dose (10 Gy) of total body gamma irradiation in Gamma cell 220 (Atomic Energy Commission, Canada Ltd). The dose rate was 0.28 rad/sec. Fresh air was continuously circulated in the irradiation chamber to avoid hypoxic conditions.


Gamma Cell 220

**Treatment groups**

There were three treatment groups used for whole study :

1. control (no treatment)- 6 animals
2. Radiation only (10 Gy)- 6 animals
3. P. hexandrum extract + Radiation (10 Gy)- 6 animals

Mice exposed to 10 Gy radiation and at various time intervals (0, 6 hr and 24 hrs), sacrificed and bone marrow cells were taken out from femur bones followed by immunostaining with Sca 1 antibody and Flow Cytometry. All mice have died after 14 days following lethal dose of radiation.

Mice were injected *P. hexandrum* extract before 1 hr of irradiation and bone marrow cells have been isolated at 14 days and 30 days post-irradiation.

**Chemicals & Reagents:**

All chemicals and reagents used for the experiments were of high quality.

**Phosphate buffer saline ( PBS): 1000 ml**

NaCl – 8.0 gm

$KH_2Po_4$ – 0.2 gm

$Na_2HPo_4$ – 1.16 gm

KCl – 0.2 gm

pH – 7.3 -7.4

All above chemicals dissolved in 500 ml double distilled water, maintain pH and volume, autoclaved it at 121˚C, 15 psi pressure.

**RBC lysis buffer** : **100 ml**

Ammonium chloride – 0.802 gm

Potassium bicarbonate – 0.1 gm

Disodium EDTA – 0.00372 gm

All above chemicals dissolved in 50 ml double distilled water and maintain volume 100 ml and stored at 4˚C.

**1% BSA-PBS : 50ml**

gm BSA (Bovine serum albumin)

Dissolved 0.5 gm BSA in 30 ml PBS and then maintain volume 50 ml and stored at 4˚C.

**70% Ethanol: 50 ml**

Dissolved 35 ml pure ethanol in 15 ml double distilled water and stored at 4°C.

**Isolation of bone marrow cells:**

All treated and control animal were sacrificed at various time intervals (0, 6, 14 and 30 days). Both femurs were excised from mice, removed muscles and bone marrow cells isolated by flushing the femur with 1 ml PBS (chilled). Centrifuged bone marrow cells at 2000 rpm for 8 min, then washed with RBC lysis buffer to lyse RBCs and again repeated the washing step. The suspended pellet contains bone marrow cells was fixed with chilled 70% ethanol (1 ml) chilled and stored at -20˚ C.

**Sca 1 staining**

Fixed cells were centrifuged at 2000 rpm for 8 min and washed with 1% BSA-PBS. Cells were stained with Sca1 (BD bioscience) at 1:50 dilution (i.e. 5 µl Ab and 245 µl 1% BSA-PBS) for 1hr at 4 C on shaker.

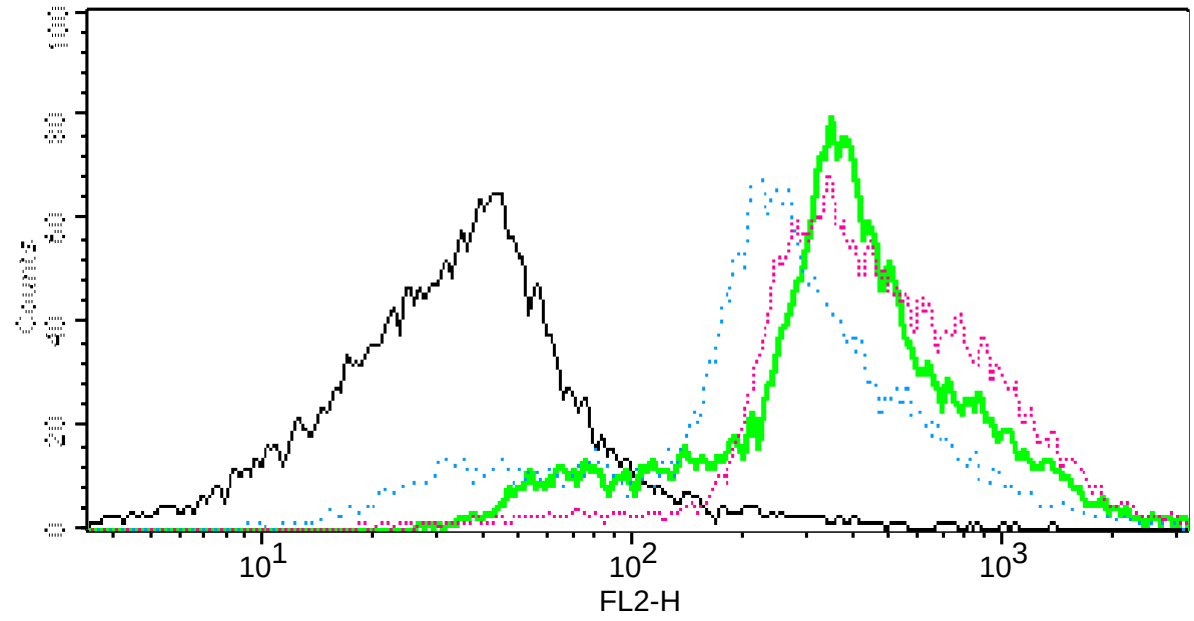
After 1hr centrifugation was done at 2000 rpm for 8 min and resultant pellet washed with 1 ml 1% BSA-PBS, thereafter, cells were processed for Sca 1 fluorescence on flow cytometry.

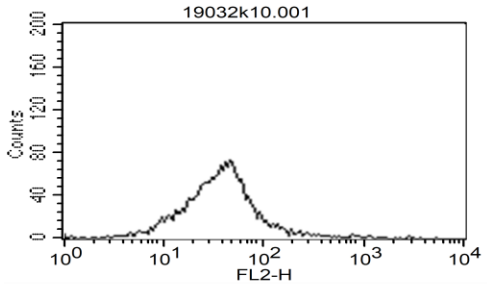
## RESULTS

**Flow cytometric analysis:**

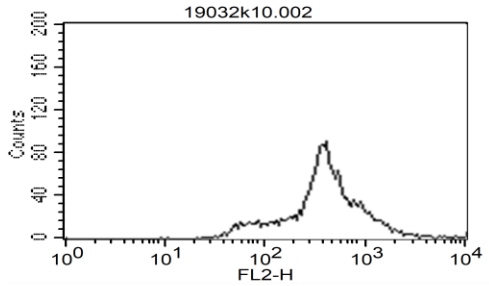
Fluorescence activated cell sorting (BD-FACS Callibur (Becton Dickenson, USA, equipped with Cell Quest pro software) have made possible to isolate and further purify many types of stem cells when used in combination with functional assays for stem cell properties. Through this tool present study has evaluated the effect of radiation on Sca-1 positive population of hematopoietic stem cells and their modulation by *P. hexandrum*. Various treatments groups which included control (no treatment), radiation only (10 Gy), drug + radiation (10 Gy) were made for whole study. 10 Gy radiation exposure to mice lead to significant reduction of Sca 1 positive population 12% and 65% at 0hr and 6 hr respectively. *P. hexandrum* treatment has significantly protected and increased 2% Sca I positive cells on 14 th day when compared with control cells. However, restoration of 66% Sca 1 positive cells was observed on 30 th day

| Samples | Mean value |
|---|---|
| Control negative | 49.54 |
| Control | 495.37 |
| *P.hexandrum* + R 14 th day | 589.54 |
| *P.hexandrum* + R 30 th day | 331.25 |

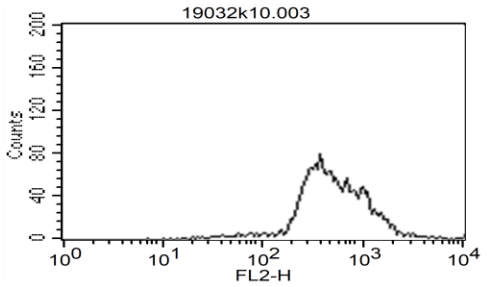


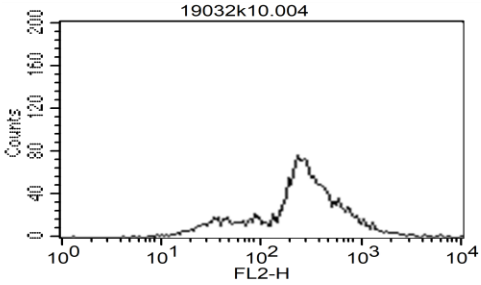

Negative control




control




*P. hexandrum* +R 14 th day




*P. hexandrum* +R 30 th day

*Radiation mediated reduction of Sca 1 positive cells and their restoration by P. hexandrum*

**Financial Support and Sponsorship:** Nil

**Conflict of Interest:** None

## REFERENCES

1. Wang X, Hu Q, Nakamua Y, Lee J, Zhang G, From A, Zhang J. The role of the Sca-1+/CD31- cardiac progenitor cell population in postinfarction left ventricular remodeling. Stem Cells 2006; 24:1779-1788.
2. Baum, C.M., Weissman, I.L., Tsukamoto, A.S., Buckle, A.M., and Peault, B. (1992). Isolation of a candidate human hematopoietic stem-cell population. Proc. Natl. Acad. Sci. U. S. A. *89,* 2804–2808.
3. Bradfute B, Graubert T, Goodell M. Roles of Sca-1 in hematopoietic stem/progenitor cell function. Experimental Hematology 2005; 33:836-843.
4. Cahwla R, Arora R, Sagar RK, Singh S, Puri SC, Kumar R, Singh S, Prasad J, Khan HA (2005). 3-o-β-D-galactopyranoside of quercetin as an active principle from high altitude *Podophyllum hexandrum* and evaluation of its radioprotective properties. *Z. Naturforschung*: 16c:728-738.
5. D. N. Haylock, B. Williams, H. M. Johnston, et al., "Hemopoietic stem cells with higher hemopoietic potential reside at the bone marrow endosteum," *Stem Cells*, vol. 25, no. 4, pp. 1062–1069, 2007
6. Ema, H., Takano, H., Sudo, K., and Nakauchi, H. (2000). *In vitro* self- renewal division of hematopoietic stem cells. J. Exp. Med. *192,* 1281–1288.
7. Goel HC, Sajikumar S, Sharma A,. (2002). Effects of *Podophyllum hexandrum* on radiation induced delay of postnatal appearance of reflexes and physiological markers in rats irradiated in utero. *Phytomedicine*; 9:447-454.
8. Gutpa D, Arora R, Garg Ap, GoelHC. (2004). Radiation protection of HepG2 cells by *Podophyllum hexandrum*. *Mol Cell Biochem;* 250:25-40.
9. Hosseinimehr Seyed Jalal. Radioprotective Agents in Radiotherapy. **27** June 2008.
10. Hoult J.R.S. and Paya M. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. Gen. Pharmacol. 1996; **27**: 713-722.
11. Ito C, Li C, Bernstein A, Dick J, Stanford W. Hematopoietic stem cell and progenitor defects in Sca-1/Ly-6A-null mice. Blood 2003; 101:517-523. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988; 241:58-62.
12. Leathwood PD, Chauffard F. Quantifying the effects of mild sedatives. Journal of psychological research, 1982/1983, **17**:115.
13. Lewis W.H. and Elvin-Lewis M.P. Medicinal plants as sources of new therapeutics. Ann. Mo. Bot. Gard. 1995; 82: 16-24.
14. Medicinal effects of ionizing radiations- second edition- Mettler-Upton,"Basic radiation physics, chemistry and biology." 1-3.

15. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman ML, Schnelder MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. PNAS 2003; 100:12313-12318
16. Sagar, R.K., Prasad, J., Singh, S., Arora, R. and Goel, H.C. (2003): Protection of Mammalian Hemopoietic System Against Lethal (10Gy) Radiation Damage by Certain Herbal Extracts: A Comparative Study. Presentation made during the XXIV Annual Conference of Indian Association of Biomedical Scientists (Theme: "Radiotracers in Drug Development") held at Laser Science and Technology Centre and Institute of Nuclear Medicine and Allied Sciences, Delhi. 1-4 Nov 2003, 87-88 (India).
17. Salin CA, Samantha N, Goel HC (2001). Protection of mouse jejunum against lethal irradiation by *Podophyllum hexandrum*. *Phytomedicine*; 8: 413-422.
18. Samantha N, Goel HC. (2002). Protection against radiation-induced damage to spermatogenesis by *Podophyllum hexandrum. J Ethnopharmacol*; 81:217-224.
19. Thomas A.David, Ognoon mungunsukh, Stephen Zins, Regina M.Day,& Michael R. Landauer "Genestein induces radioprotection by hematopoietic stem cell quiescence" int. j. radiation boil.Vol.84, no. 9, sept-2008, pp. 713-726.
20. Upadhyay S.N., Dwarakanath B.S., Ravindranath T. and Mathew T.L.. Chemical Radioprotectors. Defence Science Journal, Vol. 55, No. 4, October 2005, pp. 403-425.
21. Varanda E.A., Tavares D.C. Radioprotection: Mechanisms and Radioprotective Agents including honeybee venom. Scientific Electronic Library Online, 2005.
22. Welm BE, Teper SB, Venezia T, Graubert TA, Rosen JM, Goodell MA. Sca-1 cells in the mouse mammary gland represent an enriched progenitor cell population. Developmental Biology 2002; 245:42-56.