RADIATION DOSE RESPONSE AND DOSE MODIFICATION FACTOR OF PHYLANTHUS NIRURI ON MICE: STANDARDIZATION OF RADIOPROTECTOR EFFECT

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ABSTRACT

This study was aimed to assess the optimum route of administration for Phyllanthus niruri (family - Euphorbiaceae) alcoholic extract with maximum radioprotection by evaluating dose modification factor (DMF) and dose response on mice against different strengths of irradiation to establish the dosage profile. To optimize route of administration P. niruri alcoholic extract was administered via intraperitoneal (i.p), intramuscular (i.m), intravenous (i.v) and oral route 1 hr before 4 Gy irradiation and after 24 hr the percentage of aberrant cells were calculated. DMF was calculated by observing survival rate following whole body irradiation with 8, 9, 10 and 11 Gy radiation exposure with and without 200 mg/kg, i.p, P. niruri alcoholic extract before 1 hr of exposure. Radiation dose response effect of 200 mg/kg of P. niruri alcoholic extract was observed against 1, 2, 3 and 4 Gy gamma ray exposure by scoring different types of chromosomal aberrations from bone marrow metaphase plates. The i.p administered group showed significantly reduced aberrant cell percentage compared to i.m, oral, i.v and sham control groups. DMF was calculated to be 1.12 as evaluated by radiation LD50 determination with and without P. niruri alcoholic extract at 200 mg/kg dose. P. niruri alcoholic extract significantly (P<0.05-0.001) reduced percent aberrant cell and major aberrations like breaks, rings and polyploidy against 4 Gy radiation. The dose of 200 mg/kg was found to have maximum radioprotective potential in i.p route. It showed DMF of 1.12 with improved survival rate, delayed occurrence of lethality.
and radiation sickness. Alcoholic extract of *P. niruri* decrease the complex aberrations like rings, dicentrics and SDC indicating significant protection of bone marrow against double strand breaks and multiple chromosomal lesions. The optimum dose of *P. niruri* alcoholic extract is established to be 200 mg/kg, i.p., having safe and effective radioprotector efficiency.

**KEYWORDS:** *Phyllanthus niruri*, radioprotection, chromosomal aberration, dose modification factor, dose response, lethality.

**INTRODUCTION**

Among the several synthetic compounds tested for radioprotection, WR-2721, a phosphorylated aminothiol, is the most effective of all the compounds tested. However, its clinical application is limited due to its toxicity at the effective dose level and on repeated administration at lower doses also. There is a need to find less toxic and more effective compounds, which would be clinically acceptable. In recent years, attention has been turned towards natural products, including biological response modifiers, plant products, metal complexes, etc. as probable agents for clinical radiation protection. Several of them have shown encouraging results in animal experiments, but none of them have so far been accepted in clinical application due to different reasons.

Ayurveda, the Indian system of medicine uses plants for the treatment of various diseases. Many plants used in Ayurveda are reported to possess antioxidant and hepatoprotective properties. Endogenous antioxidants including non-enzymatic entities like glutathione, ascorbic acid and uric acid as well as enzymes like catalase, superoxide dismutase and glutathione peroxidase play a crucial role in protection against radiation-induced damage. Presence of large number of secondary metabolites having antioxidant activity helps in usages of herbs as an alternative due to their ability to combat radiation induced adverse effect. This has encouraged researchers towards search of non-toxic and effective natural compounds as radioprotectors among the Indian herbs in view of their safety and multifaceted mode of action.

*Phyllanthus niruri* (syn *P. amarus, P. fraternus* Webster, family - Euphorbiaceae) commonly known as Jar amla or Jangli amla in Hindi and Bhumyamlaki in Sanskrit. This plant is a winter weed, growing in the tropical parts of India. The whole plant, fresh leaves as well as roots are used in traditional medicine as hepatoprotective drug. Antioxidant activity and
hepatoprotective potential of *P. niruri* is reported by Harish and Shivanandappa (2006) and Sabir and Rocha (2008).²,³ Uma Devi *et al.* (2000) reported significant reduction of radiation induced micronuclei induction by methanolic extract of *P. niruri* at 25-150 mg/kg (i.p) on mouse bone marrow following whole body exposure of 4 Gy of gamma radiation.⁴ Thakur *et al.* (2011) showed that both aqueous and alcoholic extract of *P. niruri* have free radical scavenging activity at 250-500 µg/ml concentration and provided protection against radiation induced chromosomal deformity at optimal dose of 200-250 mg/kg, i.p.⁵ Phyllanthin isolated from *P. niruri* is responsible for free radical scavenging property and radioprotective effect as reported by Thakur *et al.* (2014).⁶

In all the above experiments *P. niruri* extract and active lignin compound phyllanthin was administered only by i.p. route where the dose 250 mg/kg of extract gave maximum radioprotection. To proceed further for clinical studies on human, the route of administration needs to be standardize for maximum possible efficacy without any toxicity. In this project different technique of injection, *viz.* intraperitoneal (i.p), intramuscular (i.m.), intravenous (i.v.) along with oral (p.o) administration was applied to assess the optimum route with maximum inhibition cell aberrant effect. The effect of *P. niruri* alcoholic extract was evaluated by calculating dose modification factor and its radiation dose response against different strengths of radiation to establish the dosage profile as radioprotective.

**MATERIAL AND METHODS**

**Plant material**

This is a continuation of our study on *P. niruri*. Initially *P. niruri* was collected from Bhopal, Madhya Pradesh, India, in 2005 and identified by Sr. Principal Scientist, National Botanical Research Institute (NBRI), Lucknow, with a voucher specimen (voucher no. NBRI/D/2005/495). Fresh aerial parts were collected consecutively in September 2013, air dried under shade and milled into coarse powder.

**Preparation of alcoholic extract**

Course powder (500 gm) was refluxed with 50% methanol for 2 hr at 40°C. This was repeated thrice, the supernatant was filtered and concentrated using rotary evaporator (Speed Vac SC 110A, USA) following the method of Suffness and Douros (1979).⁷ The yield was calculated to be 8.86%.
**Experimental animals**

The experiments were carried out on Swiss albino mice of both sex weighing 25-30 gm, maintained in the animal house of the Department of Research, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal, Madhya Pradesh. The animals were housed in controlled conditions of light (12:12; light : dark), temperature (22 ± 2°C) and relative humidity (55 ± 5%). The animals were housed in sanitized, sterile polypropylene cages containing autoclaved paddy husk as bedding material. They had free access to autoclaved food pallet (Golden Feed, Delhi) and filtered, acidified water. The study was approved by Institutional Animal Ethics Committee and conducted following the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg. No. 500/01/a/CPCSEA; 31/10/2001).

**Radiation exposure**

 Gamma radiation was exposed from a teletherapy unit (60Co, Cobalt, Canada) in the Department of Radiotherapy, Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal. Radiation was let vertically downwards through a collimator directed to a steel table and the field size was adjusted to 30×30 cm². The timer of the unit was preset to a calculated time for different doses of exposure. Source to surface distance was adjusted to get the required dose rate. Whole body irradiation was done restraining the unanaesthetized mice in a well ventilated perpex box (20×20×4 cm) at a dose rate of 1-11 Gy/min as per requirements of the experimental protocol from a distance of 101 cm below the source. The dose rate was calculated using the decay table provided for 60Co.

**Study protocol**

For preparation of doses alcoholic extract dissolved in 30% polyethylglycol (PEG) freshly before administration.

**In vivo study**

**Route of administration**

Mice were randomly divided into six groups of five animals in each. Group I and II; sham vehicle control and sham irradiated control mice were injected with 30% PEG, i.p. Group III-V; *P. niruri* alcoholic extract at a dose of 200 mg/kg was injected via intraperitoneal (i.p), intramuscular (i.m) and intravenous (i.v) route respectively and Group VI was administered orally. Animals were sacrificed by cervical dislocation after 24 hours of treatment and
metaphase plates were prepared from the bone marrow cells of femur and stained with Giemsa. A total of 100 metaphase plates were scored per animal. The percentage aberrant cells were calculated as the number of aberrant cells over the number of dividing cells.\textsuperscript{[8]}

**Dose modification factor**

Animals were divided into 8 groups each having 5 mice and treated as follows and then exposed with sub-lethal and lethal strength of gamma radiation. Group I-IV; radiation alone group animals were treated with 30% PEG, i.p., and whole body irradiated with 8, 9, 10 or 11 Gy respectively. Group V-VIII; alcoholic extract with radiation exposed group animals were irradiated with 8, 9, 10, or 11 Gy after an i.p. injection of 200 mg/kg of *P. niruri* alcoholic extract before 1 hr of exposure. All the animals were monitored daily up to 30 days for any toxic symptoms and mortality was recorded.

The radioprotective efficiency of a compound is generally expressed as the dose reduction factor (DRF) or dose modification factor (DMF), which is the ratio of the radiation dose to produce a given biological effect in the presence of the protector in respect to the same effect in the absence of the protector. In the evaluation of radioprotectors *in vivo*, the commonly used biological end point is mortality expressed as LD\textsubscript{50} (death of 50% of animals).\textsuperscript{[9]}

The DMF was calculated as follows:

\[
\text{DMF} = \frac{\text{Radiation LD}_{50} \text{ in the presence of the protector}}{\text{Radiation LD}_{50} \text{ in the absence of the protector}}
\]

**Radiation dose response**

Radioprotective effect of 200 mg/kg of *P. niruri* alcoholic extract was observed against different strength of gamma ray exposure. Animals were divided into 9 groups each having five mice each and treated as follows. Group I; vehicle control group was injected i.p with vehicle 30% PEG. Group II, IV, VI and VIII; sham irradiated animals were treated with vehicle and after 1 hr exposed with 1, 2, 3 and 4 Gy radiation doses respectively. Group III, V, VII and IX; animals were exposed with 1, 2, 3 and 4 Gy radiation doses respectively after an i.p. injection of 200 mg/kg of *P. niruri* alcoholic extract 1 hr before exposure.

Animals were sacrificed by cervical dislocation after 24 hr of irradiation and metaphase plates were prepared from the bone marrow cells of femur and stained with Giemsa. A total of 500 metaphase plates were scored per animal. The different aberrations, chromatid,
chromosome breaks, fragments, rings, dicentrics, polyploids, pulverized, severely damaged cells were scored under light microscope.

**Chromosome aberration scoring**

Bone marrow was flushed out with normal saline (0.85 %) vortexed for thorough mixing and centrifuged (1000 rpm) for 10 min. The supernatant was discarded and the pellet treated with 5 ml of freshly prepared 0.567% KCl solution for 25 min at 37°C. The tubes were centrifuged again at (1000 rpm) for 10 min, the supernatant discarded and the pellet was fixed in freshly prepared Carnoy’s fixative (3:1, methanol: acetic acid) for 5 hours. The tubes were centrifuged and supernatant discarded, fresh fixative was added and this process was repeated twice. After the last wash cell suspension was dropped on precleaned chilled slides using a Pasteur pipette and flame dried.

**Staining and scoring**

The slides were stained with 1% giemsa for 15-20 minutes, rinsed in clean running water and dried. They were mounted using entellan and scored under a light microscope using a 60× objective (Olympus, Japan).

The different aberrations were scored based on the classification by Savage (1999).[^10]

**Breaks:** The fragmented region of a chromosome arm, which is not associated with any exchange process. Chromatid breaks are noted only on one arm of the chromosome and chromosome breaks are when both arms of the chromosomes are involved.

**Fragments:** Chromosomes that are larger than the width of the chromatid arm. Marge para 3 and 4.

**Centric rings:** Chromosomes whose ends have joined to form a ring, including a centromere. When the centromere is absent in the ring, it is termed an acentric ring.

**Polyploidy:** Plates containing more than 2n number of chromosomes.

**Severely damaged cells:** A plate with 10 or more number of aberrations was scored as severely damaged cells (SDC) as described by Gupta and Uma Devi (1985).[^11]

**Statistical analysis**

Data were presented as Mean ± SEM of five mice and statistically compared by one way ANOVA followed by Tukey’s post test using Graph PAD Instat Software. A value of P<0.05 was considered significant.
RESULTS

Route of administration

To select the most effective route of administration, animals were given 200 mg/kg of *P. niruri* alcoholic extract by different route of administration, i.p, i.m, i.v and orally before 1 hr of irradiation (4 Gy).

The percentage of bone marrow aberrant cells was calculated after 24 hr. The i.v administered group showed high percentage of aberrant cells 32.92 ± 2.92 close to sham irradiated group 43.60 ± 3.22, which is considered not effective. The doses given by i.m and i.p was more effective with significantly reduced aberrant cell percentage, respectively 23.65 ± 2.84 and 21.03 ± 1.82 compared to 26.15 ± 2.60 of oral (Fig. 1).

Dose modification factor

Animal survival and DMF was studied by observing the animals for 30 days following radiation exposure. All the animals pretreated with 30% PEG and exposed to 11 Gy radiation died within 15-16 days after treatment. The common symptoms of radiation sickness observed were diarrhea, lack of food and water intake, loss of body weight, facial edema, ruffling of hair, epilation and tail necrosis. Radiation syndrome started as early as 6th to 8th day after irradiation. Animals suffered from diarrhea between 3rd to 6th day. A steady weight loss was observed from day one onwards and continued till their death. Ruffling of hair was seen between 6th to 8th day and facial edema from 10th to 12th days. The first death occurred on 10th day and 90% of the animals died by the day 14 after exposure. However treatment with 200 mg/kg before 11 Gy radiation exposure delayed the onset of radiation syndrome. Diarrhea started on 8th day, but animals recovered after 10th day. Loss of body weight was seen from day 3 and showed progressive decline till death. The first mortality was observed on day 12 and 50% of animals died within 20 days, and the remaining deaths occurred before 25th day. Pretreatment with the alcoholic extract not only delayed the onset of radiation sickness but also eliminated some symptoms and increased survival. In extract treated group exposed to 8 Gy radiation ruffling of hair were noticed between 9th to 10th days and no case of diarrhea was detected. Loss of body weight was observed from 3rd day onwards but regained by 15th day in the survivors. Mortality was delayed up to 16 days after exposure and 10% survivors showed recovery of body weight after 25 days (Fig. 2). DMF was calculated to be 1.12 as evaluated by radiation LD_{50} determination with and without *P. niruri* alcoholic extract at 200 mg/kg dose.
Radiation dose response

Radiation produced a significant dose dependent increase in the percent aberrant metaphase cells and different types of aberrations metaphase cells compared to the sham treated controls. Percent aberrant cells increased from 7.67% at 1 Gy to 47.11% at 4 Gy. *P. niruri* alcoholic extract treatment resulted in 61.66, 58.85, 53.42 and 43.23% reduction of aberrant cell respectively against 1, 2, 3 and 4 Gy radiation exposure. The most common aberrations occurred dose dependently are breaks and fragments, and complex aberrations like rings and dicentrics at 2 to 4 Gy doses of radiation exposure. At the higher dose of 4 Gy radiation, cells with multiple aberrations and polyploidy increased. Pretreatment with 200 mg/kg dose of *P. niruri* alcoholic extract resulted in a significant (P<0.05-0.001) decrease in the percent aberrant metaphase cells. Extract significantly (P<0.05-0.001) reduced the major aberrations like breaks, rings, SDC and polyploidy against 4 Gy radiation and occurrence of fragments against all radiation doses (Table 1).
Table 1: Effect of *P. niruri* alcoholic extract on radiation induced chromosomal aberrations following different doses gamma irradiation exposure

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>% Aberrant cells</th>
<th>Chromatid breaks</th>
<th>Chromosome Breaks</th>
<th>Fragments</th>
<th>Rings</th>
<th>Dicentrics</th>
<th>SDC</th>
<th>Poly-ploidy</th>
<th>Pulverized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>0.50 ± 0.29</td>
<td>0.27 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.47 ± 0.26</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Vehicle + 1Gy</td>
<td>7.67 ± 1.67(\text{ns})</td>
<td>2.93 ± 0.39(\text{ns})</td>
<td>0.47 ± 0.14(\text{ns})</td>
<td>4.27 ± 0.14(\text{ns})</td>
<td>0.00</td>
<td>0.00</td>
<td>0.16 ± 0.01</td>
<td>0.48 ± 0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>200 + 1Gy</td>
<td>2.94 ± 0.27 ((-61.66%))</td>
<td>1.67 ± 0.18</td>
<td>0.36 ± 0.7</td>
<td>2.21 ± 0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.84 ± 0.12</td>
<td>0.26 ± 0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Vehicle + 2 Gy</td>
<td>21.05 ± 3.26(\text{**})</td>
<td>4.92 ± 0.58(\text{*})</td>
<td>1.76 ± 0.30(\text{*})</td>
<td>12.96 ± 1.34(\text{**})</td>
<td>1.61 ± 0.14</td>
<td>1.16 ± 0.24</td>
<td>0.32 ± 0.01</td>
<td>1.60 ± 0.25</td>
<td>1.44 ± 0.91</td>
</tr>
<tr>
<td>200 + 2 Gy</td>
<td>8.66 ± 1.02(\text{a}) ((-58.85%))</td>
<td>2.46 ± 0.23(\text{ns})</td>
<td>0.90 ± 0.21(\text{ns})</td>
<td>6.28 ± 0.94(\text{a})</td>
<td>1.16 ± 0.12(\text{ns})</td>
<td>1.90 ± 0.44(\text{ns})</td>
<td>0.20 ± 0.02(\text{ns})</td>
<td>1.32 ± 0.14(\text{ns})</td>
<td>1.21 ± 0.24(\text{ns})</td>
</tr>
<tr>
<td>Vehicle + 3 Gy</td>
<td>28.06 ± 3.05(\text{***})</td>
<td>5.76 ± 0.71(\text{**})</td>
<td>2.05 ± 0.47(\text{*})</td>
<td>15.20 ± 1.61(\text{***})</td>
<td>2.18 ± 0.16</td>
<td>3.16 ± 0.82</td>
<td>1.81 ± 0.42</td>
<td>1.81 ± 0.16</td>
<td>1.68 ± 0.39</td>
</tr>
<tr>
<td>200 + 3 Gy</td>
<td>13.07 ± 2.80(\text{a}) ((-53.42%))</td>
<td>3.21 ± 0.25(\text{ns})</td>
<td>1.24 ± 0.17(\text{ns})</td>
<td>5.80 ± 0.67(\text{b})</td>
<td>1.12 ± 0.12(\text{a})</td>
<td>2.70 ± 0.45(\text{ns})</td>
<td>1.24 ± 0.22(\text{ns})</td>
<td>1.63 ± 0.2(\text{ns})</td>
<td>1.33 ± 0.2(\text{ns})</td>
</tr>
<tr>
<td>Vehicle + 4 Gy</td>
<td>47.11 ± 3.14(\text{***})</td>
<td>13.76 ± 1.87(\text{***})</td>
<td>5.18 ± 0.68(\text{***})</td>
<td>18.16 ± 1.84(\text{***})</td>
<td>3.40 ± 0.51</td>
<td>6.60 ± 1.04</td>
<td>5.15 ± 0.36</td>
<td>4.90 ± 0.70</td>
<td>4.26 ± 0.41</td>
</tr>
<tr>
<td>200 + 4 Gy</td>
<td>26.74 ± 2.88(\text{c}) ((-43.23%))</td>
<td>9.46 ± 1.03(\text{a})</td>
<td>2.16 ± 0.92(\text{a})</td>
<td>10.10 ± 1.68(\text{b})</td>
<td>2.21 ± 0.13(\text{a})</td>
<td>2.36 ± 0.40(\text{c})</td>
<td>2.61 ± 0.16(\text{c})</td>
<td>3.32 ± 0.33(\text{a})</td>
<td>2.40 ± 0.12(\text{ns})</td>
</tr>
</tbody>
</table>

\(\text{P}<0.05, \quad \text{**P}<0.01\) and \(\text{***P}<0.001\) when sham irradiated groups were compared vehicle control group. \(\text{aP}<0.05, \quad \text{bP}<0.01\) and \(\text{cP}<0.001\) when treated groups were compared with respective sham irradiated group. \(\text{ns= not significant. SDC = severely damaged cell.}\}
Figure 1: Effect of different route of administration of *P. niruri* alcoholic extract on percent bone marrow aberrant cell after 4 Gy irradiation in mice. 
i.v = intravenous, i.m = intramuscular and i.p = intraperitoneal.

Figure 2: Effect of *P. niruri* alcoholic extract on percent survival rate of mice after 30 days following different doses of sub-lethal and lethal gamma irradiation exposure.
DISCUSSION

Our earlier studies on *P. niruri* provided scientific validity for its alcoholic extract having promising radioprotective potential.\(^5\) The lignin phyllanthin isolated from alcoholic extract is one of the active phytoconstituents responsible for radioprotector activity of *P. niruri* and free radical scavenging seems to play an important role in its protective mechanisms.\(^6\) The previous studies have shown that 50 - 250 mg/kg dose of *P. niruri* alcoholic extract gave protection against radiation (against fixed radiation strength of 4 Gy) induced clastogenecity emphasizing the need to establish a safe and effective dose for repeated administration. From the results of this study dose response relationship was established for alcoholic extract of *P. niruri* as presented in Figure 3. The response was considered to be the ability to reduce the expression of aberrant cell in turn to increase the percentage of normal metaphasic cells in mouse bone marrow. The magnitude of response was proportional to dose, initially rises steeply and later become steady with increasing dose. This is a type exponential or hyperbolic dose response curve typically representing a graded radioprotective response characteristic of *P. niruri* alcoholic extract. The response level above 200 mg/kg dose becomes steady with very little increment in response with 250 mg/kg dose.

The 200 mg/kg dose with maximum efficacy was further optimized to establish the route of administration having maximum inhibition on radiation induced aberrant cell expression. The
animals were exposed to 4 Gy radiation after administration of 200 mg/kg dose by different route. The i.p route was found to be having maximum effect on reduction of radiation induced aberrant cell expression compared to i.v, i.m and oral.

Various chemical agents can alter cell response to ionizing radiation, either reducing or enhancing the cell response. Chemical agents that reduce cell response to radiation are called radioprotectors. They generally influence the indirect effects of radiation by scavenging the free radicals produced. The relative efficacies of radioprotectors are identified in preclinical studies by quantification of response to enable selection of the most promising candidate. The DMF or DRF is described as the best measure of effectiveness, which is the relative dose of irradiation required for a given effect (or end point) in the drug treated group as compared with a radiation-only group.

The optimized dose of 200 mg/kg, i.p was further tested to establish the dose modification factor which is generally expressed as radioprotective efficiency of a compound. The DMF of *P. niruri* alcoholic extract was measured by comparing the survival rate of mice given a single dose of 8, 9, 10, 11 Gy radiation with or without extract. Survival was considered as a binary endpoint and the DMF was calculated by regression analysis of radiation dose response curve with the ratio of LD$_{50}$ value. The animals were exposed to sub-lethal and lethal doses of radiation and observed for 30 days for the signs of radiation toxicity and survival rate. Whole body irradiation was used in our study which showed 100 % lethality at 11 Gy. Pretreatment with the 200 mg/kg alcoholic extract improved survival rate, delayed occurrence of lethality and the onset of radiation sickness, and also eliminated some other symptoms of radiation toxicity. DMF was calculated as the ratio of radiation dose with or without the *P. niruri* alcoholic extract causing the death of 50% of animals. DMF more than 1.0 indicates that the protection can be quantified in terms of DMF values.$^{[9,12]}$

This study describes a DMF of 1.12 by *P. niruri* alcoholic extract for improvement of radiation induced morbidity. DMF of 1.12 means that on average a 10% higher dose of radiation can be tolerated when *P. niruri* alcoholic extract is given along with radiation schedule. At 10 Gy there was at least a 3 fold increase in survival of mice given *P. niruri* alcoholic extract. However dose response curve for survival was steeply linear having chances of minimal effect at the highest and lowest tolerated radiation doses and more benefit at doses in between.$^{[13]}$ Results substantiates the radioprotective effect *P. niruri* alcoholic extract as an important step forward towards our goal to identify and develop drugs that can
delayed radiation injuries. Increase percentage survival by the *P. niruri* alcoholic extract compared to sham control following 9, 10 and 11 Gy radiation exposure indicates protection of bone marrow. Therefore experiment was conducted to study protection against bone marrow injury, using alcoholic extract at the optimum dose, 200 mg/kg, as it significantly reduced the side effects and increase the survival after 30 days of high dose radiation exposure.

X-rays and γ-radiations induce an immediate arrest of cells in the G₂ phase- called G₂ block, preventing further progressions of these cells through the cell cycle. The duration of G₂ block and mitotic inhibition increases with the increase in radiation dose. Radiation has increased all types of unstable aberrations like chromatid and chromosome breaks, formation of fragments, rings and dicentrics in a dose dependent manner. Enhancement in the frequency of chromosomal aberrations was reported earlier in the bone marrow of irradiated mice.¹⁴,¹⁵ Breaks and fragments arise from unrepaired single strand breaks. Rings and dicentrics require a more complex interaction, which involves rejoining of double strand breaks in an error prone manner.¹⁶ The injured cells attempt to repair the damage but following severe injury the unrepaired lesions of the DNA are manifested like fragmented chromosomes, which appear as micronuclei in rapidly proliferating cells.¹⁷ At radiation dose of 1 Gy simple aberrations like breaks and fragments occurred indicating the involvement of single strand breaks. At the higher doses of 2–4 Gy radiation cell showed increased occurrence of rings and dicentrics with multiple lesions and polyploidy involving more double strand breaks.

Alcoholic extract of *P. niruri* decrease the complex aberrations like rings, dicentrics and SDC against 4 Gy radiation dose indicating significant protection against double strand breaks and the multiple lesions. Synthetic compounds like 2-mercaptopyrropropionylglycine and S-2 (aminopropylamino) ethyl phosphorothioic acid, and flavonoids orientin and vicenin isolated from *Ocimum sanctum* is reported to have similar effect.¹¹,¹⁴,¹⁸-²⁰ The current results further confirm the protective effect of alcoholic extract of *P. niruri* against radiation clastogenecity reported in the earlier studies.⁴ Methanolic and aqueous extract of leaves and fruits of *P. niruri* showed inhibition of lipid peroxidation, scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical in vitro, and also in vivo by inhibition of the carbon tetrachloride induced formation of lipid peroxides in rat liver.¹⁵ The aqueous leaf extract of *P. niruri* showed inhibition of thiobarbituric acid-reactive species, DPPH radical scavenging and iron chelation effects.³ *P. niruri* alcoholic extract have rich presence of antioxidant phytochemicals like
flavonoids, lignans, polyphenols, coumarins and saponins. Alcoholic extract of *P. niruri* showed inhibition of hydroxyl radicals along with pro-oxidant and iron chelating ability. *P. niruri* may be able to scavenge hydroxyl radical, the end product of oxidative stress and can prevent auto-oxidation cascade by its established iron chelating ability.[5]

**CONCLUSION**

We have also reported in vitro antioxidant and radioprotective potential of lignin, phyllanthin isolated from *P. niruri*. Several mechanisms, including a potent antioxidant activity, stimulation of immune response and enhanced recovery of bone marrow have been suggested as possible mechanisms of radioprotection by alcoholic extract of *P. niruri*. In this study *P. niruri* alcoholic extract is established to be safe and effective radioprotector at the optimum dose 200 mg/kg, i.p. Its effectiveness and relative radiation toxicity reduction ability may be established further in preclinical and clinical studies to recommend as adjuvant for the cancer patients undergoing fractionated radiotherapy.

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