

**AN AYURVEDIC PREPARATION AMALAKYADI CHURNA  
PROTECTS AGAINST RADIATION INDUCED MICRONUCLEI IN  
MOUSE BONE MARROW**

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**Summary**

The radioprotective efficacy of the ethanolic extract of Amalakyadi churna (an Ayurvedic formulation) was studied against the induction of micronuclei in the bone marrow cells of whole body irradiated (4Gy  $\gamma$ -rays) Swiss albino mice. Amalakyadi churna (AC) is a powdered mixture of equiproportions of fruits of *Phyllanthus emblica* L (Euphorbiaceae), *Piper longum* L (Piperaceae), *Terminalia chebula* Retz (Combretaceae), roots of *Plumbago zeylanica* L. (Plumbaginaceae) and rock salt. In Ayurvedic system of medicine, this churna, is widely, used as carminative, digestive, appetizer, purgative etc. Treatment of mice with various doses ie., 30, 40 and 80mg/kg b.wt. intraperitoneally of Amalakyadi churna before exposure to 4Gy of  $\gamma$ -radiations (RT) resulted in a significant reduction of the micronucleus frequency. The highest decline in the frequencies of micronuclei was observed after administration of 30 mg/kg Amalakyadi churna. Where as, the frequency of micronuclei was approximately more than ten folds less than that of the irradiated standard control and it was restored to normal level after administration of 30mg/kg b.wt. of Amalakyadi churna. The results of the present study clearly demonstrate that Amalakyadi churna protects mice against radiation induced micronucleus (MN) formation and radiation induced decline in cell proliferation.

**Key words:** Amalakyadi churna (AC)  
Micronucleus (MN)  
Micronucleated Polychromo Erythrocytes (MPCE)  
Micronucleated Normochromo Erythrocytes,(MNCE)  
Radiation treatment (RT)

### **Introduction**

Amalakyadi churna is an Ayurvedic formulation, which is extensively used in traditional system of Indian medicine and by folk practitioner for treating all types of fever, and other common ailments (1). Due advancement of technology, man is oftenly exposing with hazardous radiations, its consequences are most complicated and complexity is at every level of anatomy, physiology, biochemistry, mutational, gene expression, etc. But the intake of antioxidant lowers the rate of oxidation stress and radiation induced morbidities and mortalities (2). So the effective treatment for such complex problems is lacking. The efforts have been continued to discover novel drugs from various sources. One such resource is Ayurvedic formulation, which is a combination of multiple herbal drugs, the active principles of such preparations interacts and gives synergistic effect. In contrary to the synthetic drugs, these plant based drugs are not associated with any side effects and holds enormous therapeutic potential to heal many diseases. Although initial research on antioxidants / targeting diseases was mostly on isolated pure compounds, but recent focus is more on natural formulations. It has been found that compounds in their natural formulations are more active than their isolated form (3).

Several research studies are clearly indicated the therapeutic uses of some of the Ayurvedic formulations, a brief review of the notable work is highlighted, such as Pippali rasayana for immunomodulatory activity in mice (4), Bhrahmirasayana for anti-inflammatory activity (5), Abana, Triphala, cystone, mentat, Chyavanaprasha have been reported to protect mice against radiation induced lethality (6-10), Amalakyadi churna for antifertility activity in male mice [11], Kalpaamrutha for anticancerous (12), acute and subacute toxicity studies (13). Brahmi rasayana for Alzheimer's disease in mice (14)

Several research groups in recent issues have clearly indicated that the presence of antioxidant, free radical scavenging activity, antiulcer, hepatoprotective, anticancerous, hypolipidemic, antimutagenic, antifertility, antimicrobial, immunomodulatory effects in the crude and isolated active principles of *Phyllanthus emblica* (15-17), *Piper longum* (18 - 24), *Terminalia chebula* (25 - 26) and *Plumbago zeylanica* (27).

Considering the important bioactivities of these individual plant ingredients of churna and churna itself in Ayurveda, folk medicines and also recent reports, moreover, clinical trials for therapeutic potentialities of Amalakyadi churna is lacking, thus interested in studying the radioprotective effect of Amalakyadi churna on 4Gy  $\gamma$  -irradiated albino mice

### **Materials and Methods**

**Chemicals:** Fetal Calf Serum (FCS) was procured from Sigma Chemical Co (St. Louis, Mo), U.S.A. Acridine orange from BDH, England, Gurr Cat No-34001. 9704640E, KH<sub>2</sub>PO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, KCl, NaCl, glacial acetic acid, methanol, ethyl alcohol from Ranbaxy fine chemicals, New Delhi, India..

**Amalakyadi Churna:** Amalakyadi churna was prepared by using standard formulation prepared by an ancient Indian Physician Sarangadhara (1300 AD ~ 1400AD), and mentioned in his treatise Sarangadhara Samhita (1)

The fruits of *Phyllanthus emblica* L. (Euphorbiaceae), *Plumbago zeylanica* L. (Plumbaginaceae) *Terminalia chebula* Retz. (Combretaceae) were collected from Sandur, *Piper longum* L. (Piperaceae) from GKVK, Bangalore (Karnataka, India) in October–November months and authenticated at the herbarium, Gulbarga University, Gulbarga. The rock salt was purchased from the local Ayurvedic shop. The plant materials and rock salt were powdered separately and sieved through muslin cloth. They were taken in equal proportions and mixed well. This Amalakyadi churna was stored in an airtight container for further processing.

The 100g of Amalakyadi churna was extracted in 90% ethanol at 50 - 60°C in a soxhlet apparatus. The extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (50 -60°C). An approximate 6.05% yield of the extract was obtained.

**Preparation of Drug:** The dried 90% of the ethanolic extract of Amalakyadi churna was taken and weighed 30mg, 40mg and 80mg and was dissolved in 10ml of distilled water, separately for further treatment

**Irradiation:** The whole body of mouse was made prostrate, immobilized, later inserted cotton plugs in the restrainer and exposed to 4Gy dose of <sup>60</sup>CO gamma radiation (Theraton Atomic Energy Agency, Ontario, Canada) in a specially designed well ventilated acrylic box. A batch of 5 mice were irradiated each time at a dose rate of 1Gy / 1.7min and 20 X 20 area and at a distance (mid point) of 50cm from the source. The exactly after 25<sup>th</sup> hour different post treatment groups of mice were analyzed for the micronucleus study, chromosomal aberration, differential leucocyte count of the femur bone marrow cells and also studied the histopathological changes in different organs of lungs, liver, spleen, kidney, stomach, intestine and heart.

**Animals:** Swiss albino mice of either sex weighing ~18g to 25g were used in the present study. Mice had free access to food and water and were maintained under 12 hours light and 12 hours dark cycles. For each experiment, micronucleus and chromosomal aberration studies, 40 mice were taken and divided them into 8 groups of 5 mice each. The I-group of mice was injected 0.2ml of double distilled water (ddw) intraperitoneally, 30 minutes prior to 4Gy  $\gamma$ -radiation which served as a respective irradiated control. The II, III and IV groups of mice were injected with the different doses of 30, 40 and 80mg/kg b.wt. of the Amalakyadi churna, intraperitoneally, exactly 30 min before irradiation. V group of mice were injected 0.2ml of ddw alone, VI, VII and VIII groups of mice were injected only with 30, 40, and 80mg/kg b.wt of the alcoholic extract of Amalakyadi churna alone, corresponding approximately 1/10<sup>th</sup>, 1/8<sup>th</sup> and 1/4<sup>th</sup>, respectively of LD<sub>50</sub> of Amalakyadi churna (ie., 313mg/kg b.wt), respectively.

**Micronucleus assay:** The above said treatment group was used. The procedure of micronucleus test described by (28) was followed. The mice were sacrificed exactly after 24 hour of post treatment period, the femoral bone marrow cells were flushed out with fetal calf serum (FCS) separately. The suspension was centrifuged, pellets were mixed with few drops of FCS. Smears were drawn on to the pre-cleaned coded slides using a drop of the resultant suspension. These slides were air dried and fixed in absolute methanol, then stained with 0.125% acridine orange. These slides were observed under fluorescent microscope (Carl Zeiss Photomicroscope III, Germany, using a 40X Neofluar Objective). A minimum of 2000 PCE, 2000 NCE, a total of 5000 erythrocyte cells were counted for each mouse. A total of 25,000 PCE and NCE were counted for each group. Data regarding to PCE/NCE ratio were also calculated.

## Results

All the doses of Amalakyadi churna provided protection to post irradiated groups of mice with varying magnitudes of potency against the radiation induced damages in femoral cells, however, the highest protection against radiation induced damages in femoral cells was observed for 30mg/kg.

**Micronucleus assay:** The treatment of mice with various doses of Amalakyadi churna for 30min before exposure to 4Gy  $\gamma$ -radiation resulted in significant reduction in the micronucleus frequency. The highest level of decline (i.e., 10.15 folds) in the frequencies of PCE bearing one micronuclei was observed in 30mg/kg+RT, there was, 7.51 and 3.1 folds decrease in the micronucleus frequency was noticed in 40 and 80mg/kg + RT groups, respectively. Similarly, 6.26, 5.95 and 3.05 folds decline of two nucleated MPCE, 20.5, 7.13 and 3.5 folds decline of three nucleated MPCE in 30, 40 and 80mg/kg+RT groups, respectively. Where as, insignificant changes were noticed in the decline of PCE bearing four micronucleated cells all drug with irradiated groups of mice, when compared with irradiated control group. The total MPCE (i.e., one + two + three + four micronucleated MPCE cells) showed significant decline i.e., 9.14, 6.35 and 3.09 folds decline in 30, 40 and 80mg/kg+RT groups respectively (Table -1)

Table-1 Effect of Amalakyadi churna on 4-Gy  $\gamma$ -radiation induced micronucleus on day-1

SI No (Treatment groups)	Dose	Frequency of MPCE $\pm$ SEM (Per 1000 cells)				Total MPCE $\pm$ SEM (Per 1000 cells)	Frequency of MNCE $\pm$ SEM (Per 1000 cells)				Total MNCE $\pm$ SEM (Per 1000 cells)	PCE/NCE Ratio $\pm$ SEM (Per 1000 cells)
		One micronucleated PCE	Two micronucleated PCE	Three micronucleated PCE	Four micronucleated PCE		One micronucleated NCE	Two micronucleated NCE	Three micronucleated NCE	Four micronucleated NCE		
01	RT	41.81 $\pm$ 4.33	4.82 $\pm$ 0.52	1.64 $\pm$ 0.50	0.26 $\pm$ 0.12	57.43 $\pm$ 6.14	3.89 $\pm$ 1.14	0.3 $\pm$ 0.12	0.07 $\pm$ 0.08	--	4.48 $\pm$ 1.40	0.46 $\pm$ 0.04
02	30mg/kg + RT	4.12 $\pm$ 1.07a	0.77 $\pm$ 0.19a	0.08 $\pm$ 0.09a	0.09 $\pm$ 0.01	6.28 $\pm$ 1.48a	1.25 $\pm$ 0.27a	--	--	--	1.26 $\pm$ 0.27a	0.64 $\pm$ 0.05a
03	40mg/kg + RT	5.57 $\pm$ 1.49a	0.81 $\pm$ 0.17a	0.23 $\pm$ 0.09a	0.00 $\pm$ 0.01	9.04 $\pm$ 1.12a	2.59 $\pm$ 0.66	0.33 $\pm$ 0.15	--	--	2.63 $\pm$ 0.87	0.62 $\pm$ 0.14a
04	80mg/kg + RT	13.53 $\pm$ 1.81a	1.58 $\pm$ 0.62a	0.47 $\pm$ 0.25	--	18.58 $\pm$ 2.53a	2.96 $\pm$ 0.64	0.28 $\pm$ 0.13	--	--	3.39 $\pm$ 0.81	0.59 $\pm$ 0.07
05	Control	2.38 $\pm$ 0.89	--	--	--	0.78 $\pm$ 0.89	0.01 $\pm$ 0.0	--	--	--	0.14 $\pm$ 0.01	0.84 $\pm$ 0.06
06	30mg/kg	2.26 $\pm$ 0.21	--	--	--	1.26 $\pm$ 0.21	0.08 $\pm$ 0.01	--	--	--	0.18 $\pm$ 0.66	0.88 $\pm$ 0.01
07	40mg/kg	2.25 $\pm$ 0.75	--	--	--	1.25 $\pm$ 0.71	0.09 $\pm$ 0.0.2	--	--	--	0.15 $\pm$ 0.63	0.90 $\pm$ 0.04
08	80mg/kg	2.43 $\pm$ 0.75	--	--	--	1.43 $\pm$ 0.75	0.07 $\pm$ 0.02	--	--	--	0.18 $\pm$ 0.14	0.79 $\pm$ 0.05

Note: a=P<0.05 (significant difference) compared 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> groups with 1<sup>st</sup> group, similarly, 6<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> groups with 5<sup>th</sup> group.

There was, significant decline (i.e., 3.11 folds) in the frequency of MNCE with single nucleated was noticed in 30mg/kg AC + RT group, where as, similar observations in decline of one nucleated MNCE were also noticed in 40 and 80mg/kg + RT groups, but difference was insignificant. However, there was no any marked reduction of two, three and four micronucleated NCE cells were noticed in different groups of drug with irradiated and irradiated alone. The total MNCE in 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups of mice showed 3.56, 1.7 and 1.3 folds decline in 30, 40 and 80mg/kg +RT groups, respectively, but the statistical difference was noticed only in 30mg/kg + RT group when compared with only irradiated group. However, there was significant decline in the PCE/NCE in irradiated group of mice. (Table-1)

The PCE/ NCE ratio was significantly higher in the different concentrations of Amalakyadi churna with 4Gy  $\gamma$ -radiation treated groups compared to the DDW + irradiated control. The pretreatment of mice with different concentration of Amalakyadi churna, in post irradiated groups of mice arrested the radiation induced decline in the PCE/NCE ratio and further, it was restored to normal level.

In 30, 40 and 80mg/kg of Amalakyadi churna alone did not produce any micronucleus of both MPCE and MNCE and also there was no changes in PCE/NCE ratio in both the drug (alone) treated and double distilled water treated (control ) groups of mice.

### **Discussion**

Whole body irradiation (4Gy- $\gamma$ -radiation) of mice resulted in sufficient increase in the frequency of micronucleated PCE and NCE. This inturn leads to the genotoxicity, reduced the mitotic index ratio, DNA damage, chromosomal mutation, and hematopoiesis in femoral bone marrow cells and improper /or non-functioning of some of the vital organs into the body of an irradiated organism. Although exposure to high doses of radiation causes DNA damage including damage to nucleotide bases, cross linkage and DNA single and double strand breaks, these changes in DNA are the principle lesions of importance in the induction of both chromosomal abnormalities and gene mutations and cancer (29-30). However, which obviously might be strongly related with oxidant status and the depletion of endogenous antioxidants in femoral cells and also increase in the level of free radicals, which mediated through reactive oxygen species and affected the cellular metabolism. Free radicals have been regarded as a fundamental cause of different kinds of diseases. They cause biochemical damage in the cells and tissues, which result in complicated diseases like diabetes mellitus, aging, cancer etc (31). Moreover, Bone marrow is one of the acute responding normal tissues in radiotherapy and chemotherapy of cancer (32). Micronucleus is formed from an acentric fragment, a whole chromosome that fails to be incorporated into daughter nuclei following mitosis due to a defective kinetochore. The assessment of these Micronuclei could also provide an indication about chromosomal aberrations (33).

The reports by several research groups in recent issues have clearly shown that, the ellagic acid, gallic acid, quercetin, kaempferol, emblicanin, flavonoids of *Phyllanthus emblica* (34), piperine, vitamin-E and A of *Piper longum* (35-36), chebulanin, casuarinin, chebulinic acid, 1, 6-di-O-galloyl- $\beta$ -glucose, chebulic acid, of *Terminalia chebula* (37) possesses strong antioxidant property and reduces the oxidation stress and other side effects caused by radiotherapy and chemotherapy. Hence, the Amalakyadi churna has significant radioprotective activity may be due to the combined effect of many of these constituents.

### **Conclusion**

Amalakyadi churna exerted the highest activity and proved as a potent and remarkable radio protective agent. In conclusion, the results of our present study clearly indicated that the 4-Gy  $\gamma$ -radiation induces significant changes in the frequency of micronuclei. The treatment of Amalakyadi could be seen through the reduction of cytogenetic damage and showed reduction in the frequency of micronuclei. This Amalakyadi churna helps to arrest and also in the restoration of radiation induced damages to nearly normal level. Obviously, Amalakyadi churna is a mixed preparation of all these useful plant ingredients, perhaps the combined effect of all these chemical groups might have synergistically exhibited the strong radioprotective potential. The mechanism of the radioprotective action of Amalakyadi churna extract in this animal model may thus be its free radical scavenging activity and its ability to protect cellular molecules from the oxidative damage.

### **Acknowledgement**

Author would like to sincerely thank Prof. Uma Devi, Scientist, Former chairman, Department of Radiobiology, Kasturba Medical College, Manipal, Karnataka, India, for providing necessary facilities and help to carryout this investigation.

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