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# Radioprotective Potential of Phycocyanin (PC) From Spirulina Platensis Against Radiation Induced Haematological Changes in Wistar Rat

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**Abstract**

Spirulina platensis, a blue green microalga, is proved for its antioxidant potential. Phycocyanin the phytopigment from Spirulina plays important role in cell protection from oxidative damage by scavenging reactive oxygen species (ROS) The therapeutic potential of Spirulina placed it as a key component in modern herbal drug development and dietary supplement. This study investigates the radioprotective potential of Phycocyanin, a phytopigment from Spirulina platensis, against gamma radiation exposure in Wistar rats. The Whole-body gamma radiation exposure at a dose 8 Gy was carried out. Analysis of post irradiated behavioural and haematological parameters shows less severe alterations and substantial recovery in phycocyanin pretreated group at a safe dose of phycocyanin 400 mg/ kg body weight within two weeks.

**Keywords:** Spirulina platensis, Phycocyanin, Antioxidant potential, Reactive oxygen species (ROS)

## INTRODUCTION

Exposure to ionizing radiations whether it is natural from sun, background radiations from earth crust, accidental radiations from nuclear reactors, and therapeutic or occupational radiations during diagnosis as well as treatment such as radiotherapy for cancer patients, poses harmful effects. Free radicles generated from various oxidative reactions as a byproduct of metabolism are one of the main reasons behind oxidative damage (M.Velco et al.2004). As these free radicals react with various biomolecules leads to alteration by the process of ionization and damage its function may result in metabolic injury. (Anjali Singh et al.2011). Radiation damage occurs through the ionization of biomolecules and water molecules within living cells, leading to the formation of free radicals. Ionizing radiations interacts with biomolecules such as proteins, nucleic acids, and lipids, particularly when exposure exceeds a critical threshold alters the biological functions. During radiotherapy where the radiation exposure is carefully controlled also results in various side effects. To overcome the limitations associated with chemical radioprotectors, the natural plant derived compounds are the phytochemicals synthesized by the plants to combat unavoidable radiation exposure from sunlight and natural background radiations.

Dose dependant radiation damages are studied by observing haematopoietic syndrome, gastrointestinal syndrome and central Nervous System Syndrome. Many phytopigments called adaptogens were tested for their radioprotective properties. Many of the natural antioxidants with their strong antioxidant property

such as vitamin C proved as a major natural radioprotector. In-vivo and In-vitro pharmacological properties of phycocyanin has been extensively studied. The current research work leads to explore the radioprotective properties of the Pigments Phycocyanin (PC) the phycobiliprotein from the algae Spirulina platensis.

**Material and Methods:** Wistar rats were used as an in- vivo system to test the phytopigment Phycocyanin from *Spirulina platensis* for its radioprotective potential. The animals were purchased from National Toxicology Centre (NTC), APT, Research Foundation, Pune, Maharashtra. The protocol was approved by Institute Animal Ethics Committee (IAEC) (approval No: RP. No. 06/2223). OECD guidelines were followed. Radiation exposure experiments were carried in the GIC facility at Government Institute of Science, Aurangabad with prior approval.

Twenty-four healthy Wistar rats, both male and female with average age of 8-12 weeks were selected. The average body weight was measured 150-200 g. with the unique identification mark on cage tag and corresponding color body marking were followed to maintain the different groups.

All the rats in the experimental groups were provided with a standard laboratory rodent diet and housed in ventilated cages with controlled humidity and temperature. Animals were acclimatized in their cages for 5 days prior to start of dosing in the experimental room after routine veterinary examination. Room temperature was maintained between 22±3°C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark. Three rats per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle and bedding of clean paddy husk. Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

**Preparation of Drug and Dose Selection**

Physical parameters of the test substance, Phycocyanin were noted for its appearance, color, texture, odor moisture solubility etc. It was dark blue color without any odor and had 100 % solubility in distilled water. The isolated Phycocyanin was proved to be nontoxic in acute oral toxicity studies and proved to have an antioxidant property in laboratory animals.

The test pigment phycocyanin was stored at room temperature. The doses selected for all four animal groups were as mentioned in the study design for test material. After Acute toxicity testing Anjali Singh (2011) by observing the $LD_{50}$ calculated by PROBIT Analysis System. The Formulation of the test material was prepared in the dose levels 400 mg/kg body wt. in distilled water.

**Study Design**

The rats were randomly assigned to four groups. Each group consisted of (n=6) rats. The four experimental groups as negative Control Group-I, Group-II, Group-III and Group IV were selected as per the table 1. Prescribed dose to respective groups of animals were given orally for fifteen consecutive days. The method was described by Anjali Singh et.al. in 2011. Radiation exposure at 8 Gy was performed by exposing whole body at a distance of 50 cm from the source. The dose rate was 185.7 Gy/min in Radiotherapy unit, after 1 hour of the administration of the last dose of phycocyanin.

**Table 1 Test Drug Dose for Radioprotective Study of Phycocyanin**

| Groups (n=6) | Treatment |
|---|---|
| **Group I (Control group)** | Received only food and Distilled water. (Unirradiated rats) |

| | |
|---|---|
| **Group II** | Unirradiated Rats given oral dose of 400 mg/Kg body wt. along with food and distilled water for 15 consecutive days |
| **Group III** | Rats Irradiated at a dose 8 Gy of γ - radiation and supplied with only food and distilled water prior to exposure. |
| **Group IV** | Rats irradiated at a dose 8 Gy of γ – radiation with a prior oral dose of PC 400 mg/Kg/day body wt. along with food and distilled water for 15 consecutive days. |

After gamma exposure, all the animals were kept under observation for the onset of post irradiation changes in body weight, weakness, lethargy, urination, defecation changes sickness and mortality etc. To measure different hematological parameters, standard techniques were employed. After irradiation, Prior to the experiments, the rats were anesthetized by inhaling 5% isoflurane until their muscular tonus relaxed. Approximately 2ml of blood was collected from the orbital sinus of the animals from each group in a heparinized vial containing 0.5M EDTA.

Collected blood samples were estimated for different hematological parameters such as red blood cell count (RBCs), WBC Count, hemoglobin (HB) levels, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), packed cell volume (PCV) etc. by hematological auto-analyzer (Mindray BC-3000Plus). All the parameters were estimated in triplicates and all values were expressed as the mean ± SEM and one way ANOVA was used to calculate P values when $p< 0.05$ were considered significant.

Haematopoietic Stem cells in the bone marrow play an important role in regenerating all blood and immune system cells. This process is, however, highly sensitive to radiation exposure. The symptoms that manifest after irradiation primarily affect the bone marrow, leading to haematological disturbances. These disturbances result in a decline in the count of various blood components, a condition known as haematopoietic syndrome. In the present study, the observation was made that gamma exposure led to the depletion of blood components. Fred and Smith (1968) stated that the damaging effect of radiation on hematopoietic stem cells is the reason behind the depletion of the erythrocyte population.

In this study, rats that were administered a single γ-dose of 8 Gy without any pretreatment showed a lack of normal regaining potential of blood components. However, rats pretreated with the aqueous extract of phycocyanin at a dose of 400 mg/Kg body weight displayed a recovery of blood components comparable to the normal state. Anjali Singh (2011) discovered analogous outcomes of erythrocyte recovery in mice pretreated with E. alba, while Verma et al. (2010) documented erythrocyte recovery through a similar treatment involving Panax ginseng.

In swiss albino mice that received a gamma dose of 3.6 Gy, Daga et al. (1995) reported a notable depletion in hemoglobin. This significant decrease in Hb% is attributed to the reduction in erythrocyte count, leakage of red blood cells (RBCs), and a lowered rate of RBC regeneration in the bone marrow.

In this study, rats that pretreated with phycocyanin were observed to exhibit a greater and faster capacity for hematopoietic regeneration compared to the control group. This observation highlights the radioprotective role of phycocyanin.

A decline in HCT (hematocrit) or PCV (packed cell volume), which measures the blood volume in red blood cells (RBCs), is indicative of hematopoietic syndrome. Singh (2006) documented the regeneration of blood components using *Emblica officinalis* (Linn.) treatment. Similarly, this study recorded analogous observations with phycocyanin from Spirulina platensis. Samarth and Kumar (2003) reported a decrease in the count of leukocytes due to gamma exposure. In this context, phycocyanin was observed to safeguard

leukocytes (white blood cells or WBCs) and expedite their rapid recovery in peripheral blood tissue.

**Results**

Any toxic effect or mortality was not observed during Acute Oral Toxicity Testing. Dose up to 2000 mg/kg body weight was proved to be safe. The dose selected; 400mg/kg body weight was the 1/5$^{th}$ of the safe dose.

**Table 2: Comparative haematological parameters after receiving dose 8 Gy(Whole body)**

| Haematological parameter | Group-I | Group-II | After 7$^{th}$ day of Irradiation (Dose 8 Gy) | | After 14$^{th}$ day of Irradiation (Dose 8 Gy) | |
|---|---|---|---|---|---|---|
| | | | Group III | Group IV | Group III | Group IV |
| RBC($10^6$/mm$^3$) | 8.49± o.061 | **8.56± 0.053** | 6.32± ** ±0.091 | 7.31±* ±0.811 | 8.09 ±0.092 | 8.63 ±0.102 |
| WBC($10^6$/mm$^3$) | 19.28 ±0.043 | 19.34 ±0.031 | 8.52*** ±0.082 | 10.73*** ±0.101 | 9.61*** ±0.082 | 13.23*** ±0.103 |
| Hb (gm/dl) | 15.4 ±0.023 | 15.9 ±0.031 | 6.86*** ±0.098 | 9.24*** ±0.076 | 10.93** ±0.113 | 13.25*** ±0.087 |
| HCT(%) | 38.53 ±0.092 | 39.14 ±0.0142 | 24.62*** ±0.011 | 30.67** ±0.056 | 29.43** ±0.098 | 33.23 ±0.112 |
| MCV | 43.28 ±0.023 | 43.42 ±0.011 | 40.37 ±0.098 | 41.21* ±0.013 | 41.1 ±0.036 | 42.72 ±0.042 |
| MCH(pg) | 18.01 ±0.106 | 18.23 ±0.059 | 15.52** ±0.109 | 16.54* ±0,083 | 15.74* ±0.013 | 17.89 ±0.045 |

All values expressed as mean ±SEM***very high Significant($p<0.001$)
Highly Significant ($p<0.01$)* Significant ($p<0.05$)

All the animal groups were keenly observed for their behavioral changes after irradiation considered as a radiation syndrome. Haematopoietic Stem cells in the bone marrow play an important role in regenerating all blood and immune system cells. This process is, however, highly sensitive to radiation exposure. The symptoms that manifest after irradiation primarily affect the bone marrow, leading to haematological disturbances. These disturbances result in a decline in the count of various blood components, a condition known as haematopoietic syndrome.

Hematological changes are summarized in Table 2 Unirradiated Group II showed no significant changes ($p<0.05$). While the animals in Group III treated with single dose of 8 Gy γ - irradiation without any prior dose of phycocyanin recorded highly significant decrease ($p<0.01$) in all hematological parameters, which was highly significant. Group IV which was prior treated with phycocyanin aqueous extract 400 mg/Kg body weight, after irradiation at 8 Gy observed for less alterations in all hematological parameters as compared to the group III animals. All the parameters have been significantly regained after two weeks in the group pretreated with phycocyanin.

**Discussion**

In the present study, the observation was made that gamma exposure led to the depletion of blood components. Fred and Smith (1968) stated that the damaging effect of radiation on hematopoietic stem

cells is the reason behind the depletion of the erythrocyte population. (Fred S.S, 1968) In this study, rats that were administered a single γ-dose of 8 Gy without any pretreatment showed a lack of normal regaining of blood components. However, rats pretreated with the aqueous extract of phycocyanin at a dose of 400 mg/Kg body weight displayed a faster recovery of blood components comparable to the normal state. (Anjali Singh, 2011) discovered analogous outcomes of erythrocyte recovery in mice pretreated with *E.alba*, while Verma et al. (2010) documented erythrocyte recovery through a similar treatment involving Panax ginseng. (Verma P, 2011)

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A decline in HCT (hematocrit) or PCV (packed cell volume), which measures the blood volume in red blood cells (RBCs), is indicative of hematopoietic syndrome. Singh et.al. (2006) documented the regeneration of blood components using *Emblica officinalis* (Linn.) treatment. (Singh I, 2006) Similarly, this study recorded analogous observations with phycocyanin from Spirulina platensis. Samarth and Kumar (2003) reported a decrease in the number of leukocytes due to gamma exposure (Samarth R.M, .(2003))

## Conclusion

Observations and Results in this studies can be concluded as rats that were pretreated with phycocyanin observed to exhibit a greater and faster capacity for hematopoietic regeneration compared to the control group. This observation highlights the radioprotective role of phycocyanin. In this context, phycocyanin was observed to safeguard leukocytes (white blood cells or WBCs) and expedite their rapid recovery in peripheral blood tissue. Thus the PC the phycobiliprotein from Spirulina platensis is a member of natural radioprotector category and will have a promising role in radioprotective drug development.

## References

1. Anjali Singh, Ravish Kumar,Nivedita,J.K.Singh,Tanuja (2011): Radioprotective effect of Eclipta alba(L.)against radiation induced hematological changes in Swiss albino mice; Journal of Natural Products. Vol 4(2011:)177-183.
2. Arena V, (1971): Ionizing radiation and life. St.Louis, Mosby, Saint Louis
3. Arena V. (1971): Ionizing Radiation and Life ; St Louis, Mosby, Saint Louis
4. Broerse J.J, Mac VittieT.J,(1984): Response of Different Species on Total Body irradiation Amsterdam: Martin Nyhoff;pp 235
5. Daga S.S,Jain V.K,Goyal P.K, 919950:Radioresponse to leucocytes in peripheral blood mice against gamma irradiation and their protection by Liv.52,Probes.34(3),222-226
6. Dorge, W. (2002 ). Free Radical in the Physiological Control of Cell Function . physiology Rev,vol 82, 50-72.
7. Fred S.S,Smith,W.W,(1968) : Induced Changes in the Translatability of Haemopoietic Colony Forming Cells.Proc.Soc
8. Harbone J.B, (1998): Phytochemical Methods- A Guide to modern techniques of plant analysis 2nd Chapman and Hall Ltd, London and New York,pp.125
9. Ismail I.S, Abdel A, (2000) : Haematological and Biochemical study on rabbit post whole body X-

irradiated and treatment by Nigella Sativa oil J. Pest Cont & Environment Sci 8(1):pp15-30
10. M. Velko, M. I. (2004). Role of oxygen radicals in DNA damage and cancer incidence; . Mol.Cell Biochem 266 , 37-56.
11. Redpath J.L,Wilson R. I, (1973): Reducing compounds in radioprotection and radiosensatization : model experiments using ascorbic acid : Int. J. of Radiat, Biol 23:pp51-65.
12. Samarth R.M, Kumar A.,(2003): Radioprotection of Swiss albino mice by plant extract Mentha piperita (Linn).J Radiat Res., 44: pp 101-109
13. Sarma L,Kesavan P.C,(1993) : Protective effects of vitamin C and E against radiation- induced in vivo chromosomal damage in mouse; Int Journal of Radiation Biol.,63; pp 759-764.
14. Singh I, Dhanraj, Goyal P.K,(2006): Emblica officinalis (Linn)fruit extract provides protection against radiation induced hematological and biochemical alterations in mice. J. Environ.Pathol.Toxicol.Oncol.25 (4): 643-654
15. Vaishali Wadekar,Ajay Irmale, Kalpana Kulkarni (2017): Oxidative Stress Management in Living Cells; International Journal of Research and Analytical Reviews (IJRAR). Volume 4, Issue 4(2017) 415-424
16. Verma P, Sharma P, Parmar J, Sharma P, Agrawal A, Goyal P.K.(2011): Amelioration of radiation induced hematological and biochemical alterations in Swiss albino mice by Panax ginseng extract. Integr Cancer Ther 10 (1): pp77-84.