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RESEARCH ARTICLE

RADIOPROTETCIVE EFFECT OF METHANOLIC EXTRACT OF *Pleurotus florida*

J.RAJESH SINGH[1] AND P.K.SENTHILKUMAR[2]

[1]Assistant Professor in Biotechnology, Annamalai University, Tamil Nadu, India.
[2]Assistant Professor in Microbiology, Annamalai University, Tamil Nadu, India.
Corresponding Author: rajgenes@yahoo.co.in

Abstract

The result of studies have demonstrated significant role of radio protective activity of methanolic extract of *Pleurotus florida*. The administration of *Pleurotus florida* 50% survival in mice against a cry whole body irradiation. Untreated mice suffered 100% motality. The radiation induced losses in the body weight / bone marrow cellularity and total by *P.florida* extract treatment radiation protection by *pleurotus florida* extract at cellular and subcellular level reflects both whole some effect of scavenging of radiation induced free radicals and the repair of damaged targets and molecules. The GSH level was enhanced to normal level in animals treated with *pleurotus florida*. In last decade natural herbal products have received significant attention owing to their utility in traditional system of medicine. This could explain the radio protective activity of *pleurotus florida* extract.

Key words: *Pleurotus florida,* body weight,/ bone marrow cellularity, GSH level.

Introduction

Malignant neoplasm is a major cause of death that threatens the mankind. Genetic alternation when induced actively but without killing the cell can result in neoplasia. In cancer or neoplasia the normal cell proliferation controls are lost at the level of cell signaling, cell cycle arrest or differentiation. The uncontrolled proliferation of cells within the tissues produces cell masses known as tumors. Tumors may be benign or malignant. Cancer is the common term for all malignant tumors.

Several methods exist for the treatment of cancer in modern medicine, which includes chemotherapy, radiotherapy and surgery. Immunomodulators are being used in cancer therapy either in combination with chemotherapy or after chemotherapy and radiation therapy. All these forms of treatments are found to effect the host normal cells also (Pierce, 1998).

Radiotherapy is the most common modality for treating human cancers. Radiations is most toxic proliferating cells, higher doses are required to kill cells that are capable of proliferating, but are not actively dividing at the time of exposure. Radiation therapy evolved as a treatment of cancer because it permitted tumor eradication with preservation of normal tissue functions. Mammalian cells are most sensitive to radiation-induced damage in the late G_2 and M phase of the cell cycle (Parker, 1990).

The era of modern chemotherapy started in 1948 with the introduction of nitrogen mustard. The purpose of treating cancer with chemotheraputic agents is to prevent cancer cells from multiplication, invasion and metastasis in the host. Since cell growth and multiplication is characteristics of cancer chemotherapeutic agent must possess marked growth-inhibiting or

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controlling effect on the cancer cells and minimal or no toxic effect on the host in most effective chemotherapeutic regimens, the drugs are capable of not only inhibiting but also completely eradicating neoplastic (tumour) cells while sufficiently preserving normal cells. There is clearly a need for the discovery of new clinically efficacious agents(Baserga, 1981).

Surgery is the oldest treatment for cancer and until recently, was the only treatment that could cure patients with cancer. The major role of surgery in the diagonsis of cancer lies in the acquisition of tissue for exact histologic diagnosis.

Ionizing radiation causes damage to living tissues through a series of molecular events, such as photo electric, Compton and Auger effects, depending on the radiation energy. Human tissues contain 80% water, the major radiation damage is due to the aqueous free radicals, generated by the action of radiation on water. The free radicals react with cellular macromolecules such as DNA, RNA, protiens, membrane, etc and cause dysnfunction and mortality (Dische,1985).

The major drawback in cancer therapeutic method is suppression of immune system. Drugs that could alleviate these side effects will be highly useful in cancer therapy. An attractive way to achieve this would be to induce an immune response. Immunomodulators are materials which can modify body's defense mechanism. Use of plants as a source of immunomodulators is still in a primitive stage (Maunch *et al*, 1995).

Immunomodulators work mainly by increasing macrophage activity. Currently it is known that many mushroom like *Grifola frondosa, Ganoderma lucidun, Lentinus edode* have shown the ability to stimulate and strengthen immune systems. Among the mushroom immunomodulators investigated, the most effective is lentinan, which is able to increase host resistence against various kinds of cancer and infectious diseases(Yoshinos *et al*, 1989).

Mushrooms are nutritionally functional food and source of physiologically beneficial and non-toxic medicines (Wasser and Weiss, 1999). Many of the medicinal mushrooms have also been shown to modulate immune system and to possess antitumor, antimicrobial, anti inflammatory activities. Attempts have been made in many parts of the world to explore the use of mushrooms and their metabolites for the treatment of a variety of human ailments (Jong and Birmingham,1992). *Pleurotus* species are wide spread through out the hardwood forest of the world. A number of *Pleurotus* species are well known for their medicinal properties such as a antiviral, anti-inflammatory, antibiotic and hypocholesterolemic activities(Nina Gude Cimerman).

Developments of effective radioprotective agents with least side effects is a compelling urgency because radiotherapy is increasingly found useful for cancer treatment. Although a large number of biological properties and medicinal uses use of *Pleurotus* species have been reported, no attempt has been made to evaluate the radio-protective effects of this medicinal mushroom. In this dissertation, result of the investigations carried out on the radioprotective effect of the methanolic extract of fruiting bodies of *Pleurotus florida,* occurring in South India, are reported.

Materials and Methods

Preparation of the extract

Fruiting bodies of *Pleurotus florida* were collected from the small scale cultivation units. The sporocarps were cut into small pieces, dried at 40-50° C for 48 hrs. and powdered. Two hundred gram of the powdered materials was extracted with petroleum ether. The defatted material was

IJCRCPS

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extracted with 70% methanol for 8-10hrs. using Soxhlet apparatus (Suffness and Durous, 1979). The solvents were completely evaporated at 40°C using rotary vacuum evaporator. The residues thus obtained was designated as methanol extract. Methanol extracts was dissolved in distilled water to form a uniform suspension, and used in the experiments.

Animals

Female Swiss albino mice12 weeks old, (weighing 25±2g) were maintained on standard food pellets (Lipton India) and water *adlibtum*.

Treatment

Desired doses of the extract (1000mg/kg or, 500mg/kg) were administered for 5 days orally prior to whole body gamma radiation.

Irradiation

Whole body irradiation was given through Cobalt 60 gamma cell (model 220-Atomic Energy of Canada).

Experiment design

Experiment: I

Heamatological Studies

36 animals were divided into 4 groups of 6 animals.

Group I (Normal)- Distilled water was given for 5 consecutive days orally.

Group II (Control)- Distilled water was given for 5 consecutive days orally and whole body irradiated with 9 Gy of gamma rays.

Group III- 70% methanol extract of *P.florida* (1000mg/kg) was given orally for 5 consecutive days and then whole body irradiated with 9Gy of gamma rays.

Group IV- 70% methanol extract of *P.florida* (500mg/kg) was given orally for 5 consecutive days and then whole body irradiate with 9Gy of gamma rays. Hemoglobin, Total leukocyte count were studied in blood samples drawn by tail bleeding.

Blood was drawn by tail bleeding before and after (24hrs, 3rd day, 5th day, 7th day, 9th day) radiation.

Total count – WBC

380 µl of dilution fluid was mixed with 20 µl blood, after 2-3 minute, mixed gently and loaded on a heamocytometer. Total no of cells/mm^3

$$= \frac{\text{No. of cells counted x dilution factor x depth factor}}{\text{Area counted}}$$

$$= \frac{Nx20x10}{4}$$

$$=Nx50$$

Dilution factor = 1/20
Depth factor = 1/10
Cells/mm^3 = Total cells x 50 x total no of cells in 1ml blood
Area counted = 43 q mm.

Hemoglobin (Hb)

0.02 ml sample was added to 5ml of cyanoment reagent. After mixing well, the tubes were kept at room temperature for 5 minutes for incubation, optical density (O.D) was measured using reagent as blank at 546 nm.

Calculation

$$\text{Heamoglobin gm/dl} = \frac{\text{O.D of sample x N x .251}}{\text{.OD standard}}$$

IJCRCPS

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where N = Conc. of standard = 60 mg/dl

Experiment II bone marrow cellularity

72 animal were divide into 4 groups of 18 animals

Group I (Normal)

Distilled water was given for 5 consecutive days orally.

Group II (Control)- Distilled water was given for 5 consecutive days orally and whole body irradiated with 9 Gy of Gamma rays.
Group GroupIII - 70% methanol extracts of *P.florida* (1000 mg/kg was given orally for 5 consecutive days and then whole body irradiated with 9 Gy of gamma rays.

Group IV 70% methanol extracts of *P.florida* (500mg/kg) was given orally for 5 consecutive days and then whole body irradiation with 9 Gy of gamma rays.
animals were sacrificed before and after (24 hrs, 3rd day, 5th day and 7th day) radiation. Bone marrow cell were collected from femur into Phosphate buffer saline containing goats serum and was counted using heamocytometer.

Experiment III : survival study

Group I (Normal)- Distilled water was given for 5 consecutive days orally.

Group II (Control)- Distilled water was given for 5 consecutive days orally and whole body irradiated with 9 Gy of gamma rays.
Group III - 70% methanol extracts of *P.florida* (1000mg/kg) was given orally for 5 consecutive days and then whole body irradiated with 9 Gy of gamma rays.

Group IV - 70% methanol extracts of *P.florida* (500mg/kg) was given orally for 5 consecutive days and then whole body irradiate with 9 gy of gamma rays.

Animals were observed for survival daily up to 30 post-irradiation days. Data are presented as % survival after the expiry of 30 post-irradiation days.

Experiment IV : Intestinal protection

Group I (Normal)- Distilled water given for 5 consecutive days orally.

Group II (Control)- Distilled water was given for 5 consecutive days orally and whole body irradiated with 9 Gy of gamma rays.

Group III - 70% methanol extracts of *P.florida* (1000mg/kg) was given orally for 5 consecutive days and then whole body irradiated with 9 Gy of gamma rays.

Group IV - 70 % methanol extracts of *P.florida* (500mg/kg) was given orally for 5 consecutive days and then whole body irradiated with 9 Gy of gamma rays.

Animals were sacrificed on 9th day after irradiation and the intestinal mucosa was collected and GSH was determined.

Determination of tissue reduced glutathione (GSH)

Reduced glutathione in the tissue was determined according to the method of Moron et al, (1979)

IJCRCPS

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Table.1 Radioprotective effect of *p. florida extract* on gastrointestinal mucosal reduced glutathione

Groups	GSH Nano mole/mg protien
Normal	31.57±1.95
Control	17.73±2.6
P. florida+Irradiation	36±8.83
P.florida+Irradiation	27.65±5.65

Figure.1 Radioprotective effect of G.lucidum on whole body radiation induced bonemarrow cellularity

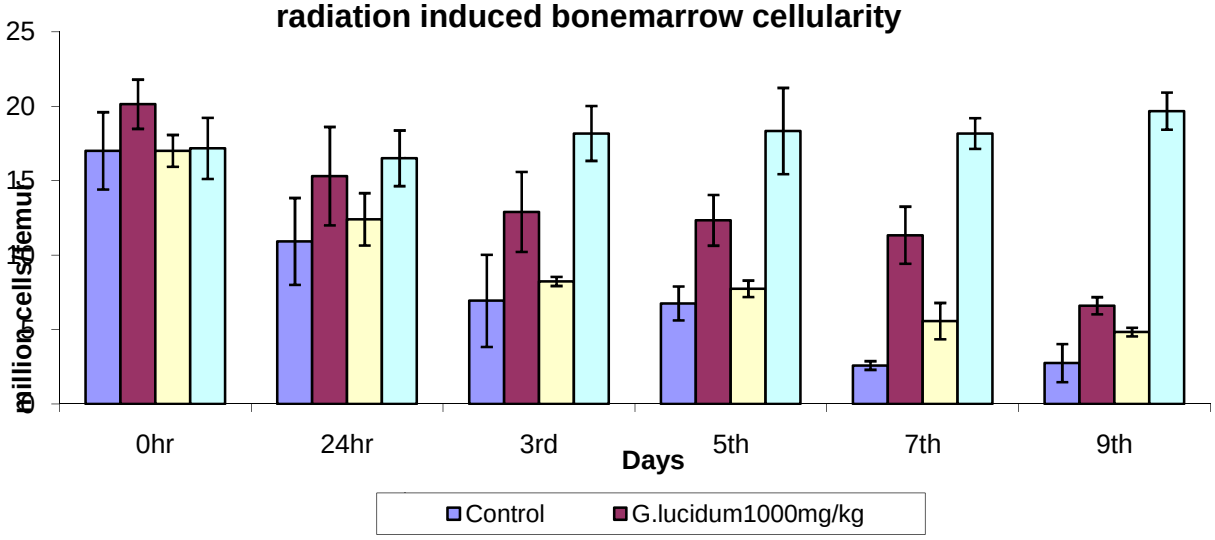

Fig.2 Radioprotective effect of P.florida extract on bonemarrow cellularity

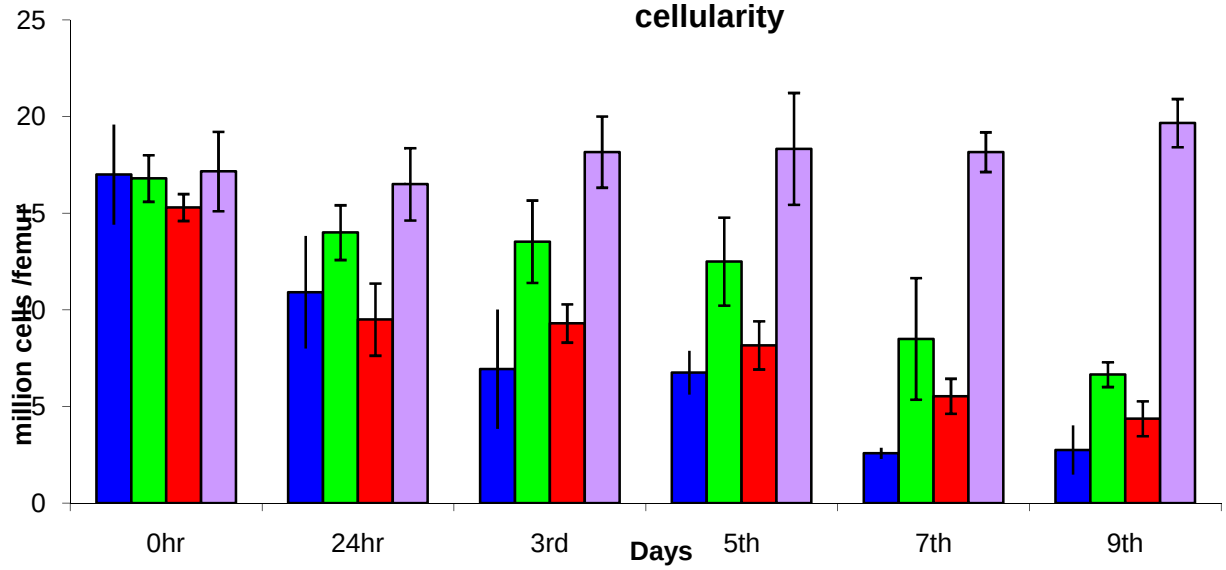

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FIG.3 Radioprotective effect of *P.florida* extract changes in total leucocyte count

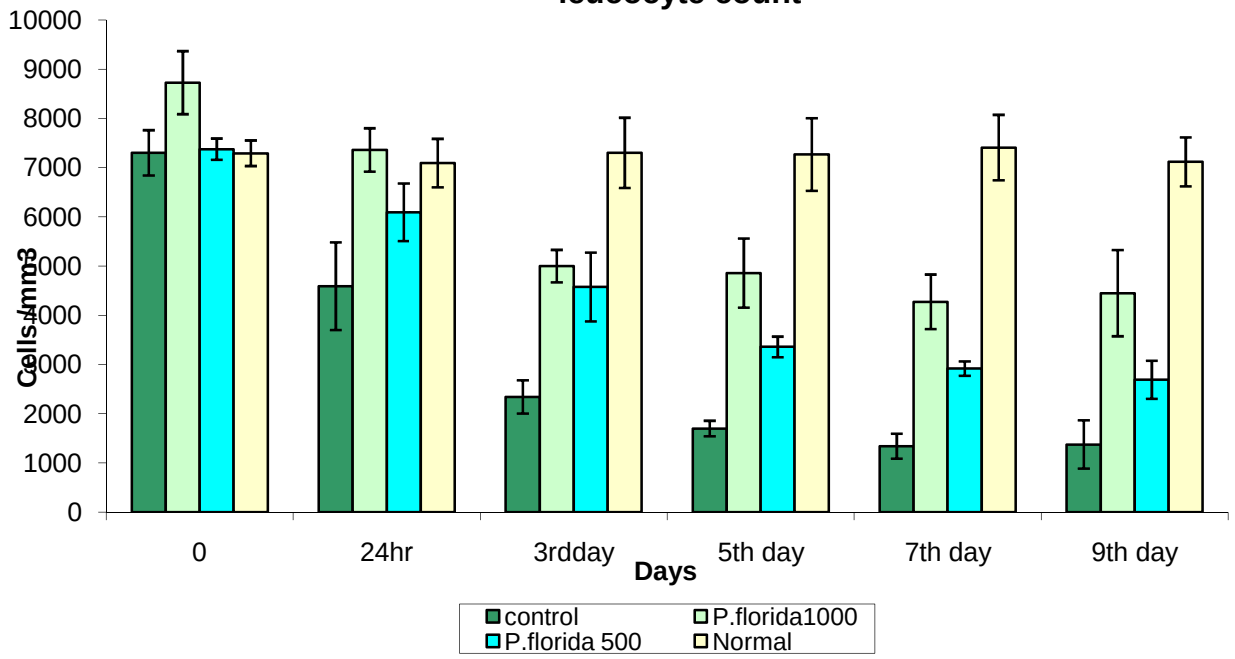
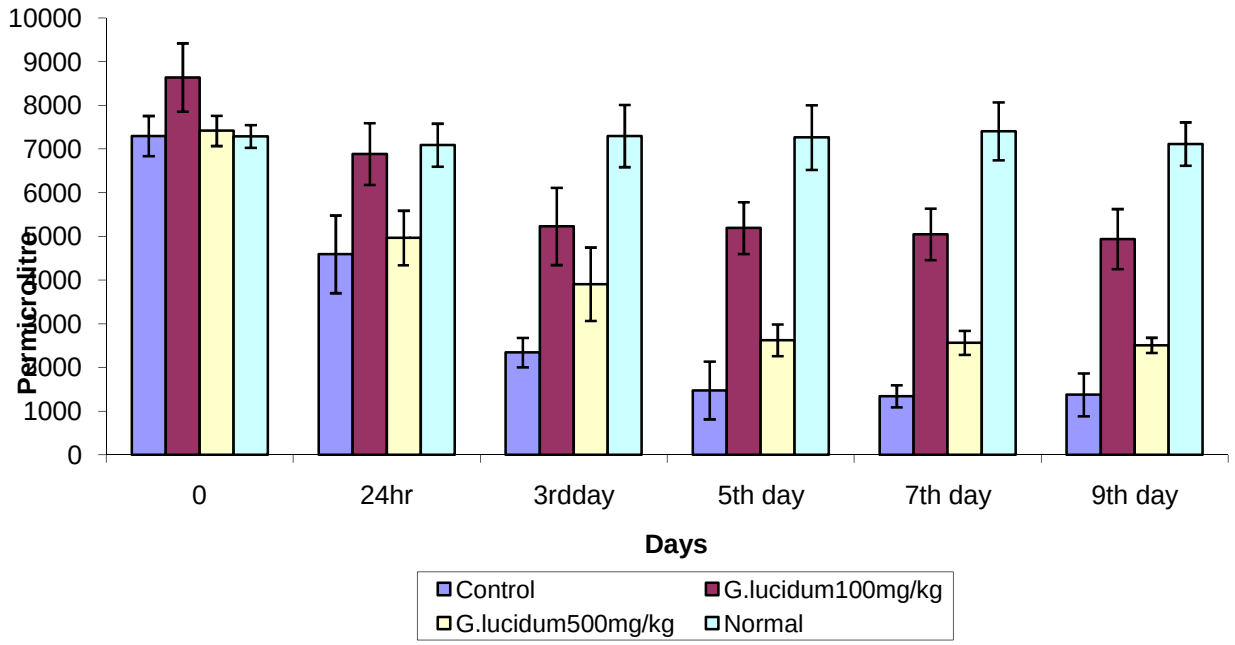


Figure.4 Radioprotective effect of G.lucdum on whole bodygamma irradiation induced changes in total leucocyte



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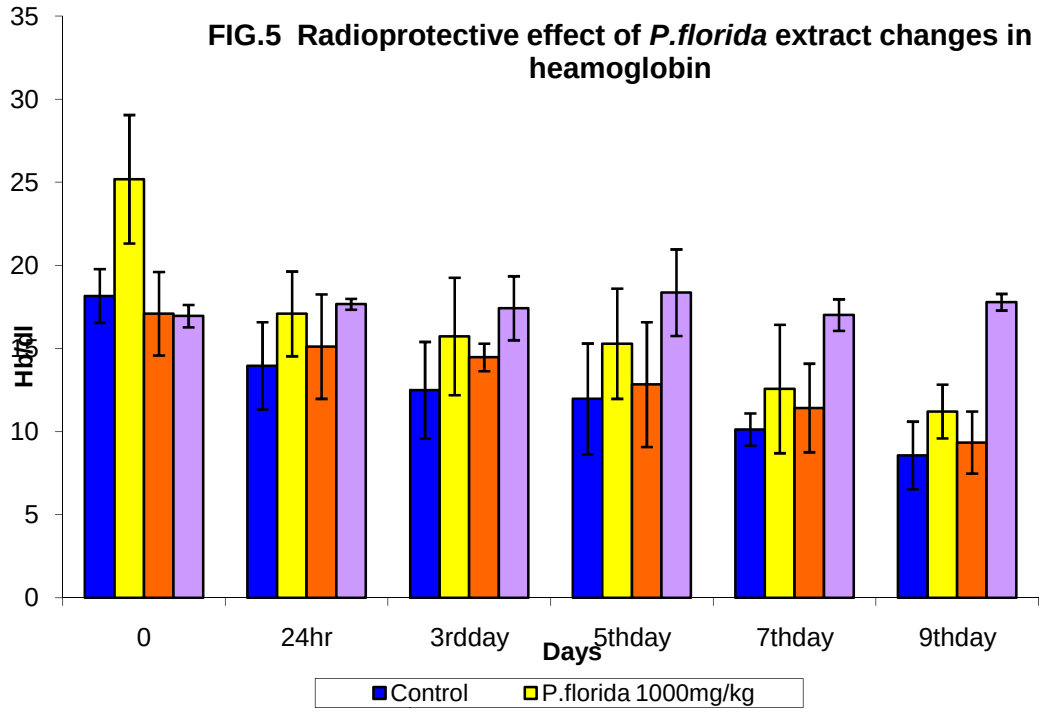

FIG.5 Radioprotective effect of *P.florida* extract changes in heamoglobin

Procedure.

0.5ml of the tissue homogenate was mixed with 0.1ml of 25% TCA and kept on ice for few minutes. These were then subjected to centrifugation at 3000g for few minutes to settle the precipitate. 0.3ml of the supernatant was mixed with 0.7 ml in 0.2m sodium phospate buffer (P^H8) and 2ml of 0.6mM DTNB (prepared in 0.2m buffer, P^H 8). The yellow color obtained was measured after 10 min at 412nm against a blank which contained .0.1 ml of 5% TCA in place of the supernatant. A standard graph was prepared using different concentrations (10-50 n moles) of GSH in 0.3 ml of 5% TCA. The GSH content was calculated with the help of this standard graph and expressed as a nanomole/mg protein.

Determination of tissue protein

Protein content in the tissue was determined according to the method of Lowry *et al*.,(1951).

0.1ml of the homogenate was mixed with 0.990 ml of distilled water, 5 ml of alkaline $CuSO_4$ (0.5 % $CuSO_4$ in 1 % sodium potassium tartrate and 2% Na_2CO_3 in 0.1 N NaOH mixed in the ratio 1:50) kept for 10 min at room temperature. 0.5 ml of 1 N Folin Ciocalteau reagent added and absorbance was measured after 20 min at 660 nm against the reagent blank. Protein content was calculated from the standard graph prepared using different concentrations (0.1-0.5 mg/ ml) of bovine serum albumin (BSA).

Results and Discussion

Experiment-I- Survival studies

Survival of animals up to 30 days of post irradiation days is shown in FIG-1. 30 days after irradiation survival of 50% animals was achieved by the administration of 1000mg/kg of *P.florida* extract.
Experiment-II bone marrow cellularity

Irradiation of animals with 9Gy resulted in decrease of bonemarrow cells from

18million cells to 2million cells in the irradiated group. Treatment with *P.florida* extract the bone marrow count was maintained above 5million cells (FIG-II) nine days after irradiation.

Experiment-III- Heamatological parameters

rradiation of animals with 9Gy resulted in decrease of total leucocyte count to 2000cells per millimeter cube, where as in the *P.florida* extract treated group the total leucocyte count remained above 4000 million cells per metre cube. (FIG-III) Heamoglobin content was also reduced by irradiation. However, treatment with *P.florida* extract the heamoglobin was maintained near normal on the 9th day after irradiation (FIG-IV)

Experiment-IV – Effect on gastrointestinal damage

Irradiation of animals resulted in decrease in GSH levels. The level of GSH in irradiated group was almost half of the normal level. The GSH level was enhanced to normal level in animals treated with *P.florida* extract. (Table-1)

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