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Modulatory Effects of *Carica papaya* (Linn.) on Electron Beam Radiation Induced Hematological Suppression and Biochemical Alterations in Swiss Albino Mice

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Abstract

The present study was undertaken to evaluate the leaf extracts of *Carica papaya* (Linn.) on hematologic and biochemical changes occurring due to irradiation of mice to sub-lethal doses of Electron Beam Radiation (EBR). The immunomodulatory response of *C. papaya* (Linn.) extract to the radiological effects by determining the levels of interleukin-6. The serum total antioxidants, lipid peroxidation and the biochemical changes in liver and kidney function were assessed. Reduction in the hematological parameters like hemoglobin, RBC, WBC and platelets were seen in the irradiated groups compared to the control group. The elevated serum malondialdehyde levels indicate increased lipid peroxidation and reduced glutathione levels and total antioxidant capacity suggest prominent oxidative stress in the irradiated groups. *C. papaya* (Linn.) (pre-treatment + radiation) treated groups have shown significant improvements in the hematological parameters. Reduction in the malondialdehyde levels and increased reduced glutathione levels indicate amelioration of the oxidative stress. There is decrease in the cytogenetic damages in the *C.papaya* (Linn.) (pre-treatment + radiation) treated groups compared to the radiation control. Significant changes were seen in the interleukin-6 levels of *C. papaya* (Linn.) (pre-treatment + radiation) treated groups indicate enhanced immune response compared to the irradiation control groups. The results indicate that the *C. papaya* (Linn.) aqueous extracts play a protective role in radiation induced hematological and antioxidant suppression. Also it enhances membrane stabilization and reduces cytogenetic damages.

Keywords: *Carica papaya (Linn.), Electron Beam Radiation (EBR), Antioxidant, Oxidative stress, Radiation protection, Membrane stabilization, Interleukin-6*

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Introduction

Exposure to ionizing radiations causes deleterious effects on physiology, metabolism and behavior in almost all the organism. The effects depend on the intensity and degree of penetration of the ionizing radiation (Storer, 1966). The study of these effects and novel approaches to counter them is of great importance in the field of radiation biology. The effects can be acute or chronic that can cause irreversible damages to the cells and tissues. The direct effects include nausea, vomiting, diarrhea, bone marrow suppression, gastrointestinal syndrome, central nervous system syndrome leading to coma and death of the organism. The chronic effect involves oxidative stress and change in the genomic stability which in turn affects the metabolic functions of the cell (Maurya et al., 2006).

In the field of medicine using radiation treatment against cancer, also bring about radiological changes in normal tissues. Thus various attempts have been made to increase the radiation sensitivity of cancerous cells and/ or protect the normal cells from radiation. Thus the use of chemicals along with radiation has proved to be ameliorative in cancer treatment (Upadhyay et al., 2005). The chemicals which protect the cells from radiation induced damages are called as 'radiation protectors' or 'radioprotectors'. The search for novel and ideal radioprotectors has been ongoing for several decades. The currently available ones are highly toxic and expensive. Hence herbal formulations which are non-toxic and inexpensive have been evaluated for their radioprotective efficacy (Suzen, 1999). Recent studies attempts to reveal the mode of action of these herbal extracts and identify the specific bioactive component which produces the protective effect. Most of these herbal extracts possess great antioxidant potential. The ability to scavenge the free radicals generated in cells due to radiation by these antioxidants present in the extracts could be the promising protective agents against radiation induced damages (Nair et al., 2001). As we have an enormous variety of flora, there is significant possibility of developing an efficient radioprotector from these natural resources.

Carica papaya (Linn.) is known to possess various biomedical applications. There are a number of varieties cultivated for various purposes. The Coorg honey dew also known as

'Madhubindu' is commonly cultivated for table as well as processing purposes. The other varieties include solo, Pusa dwarf, Pusa giant, Pusa majesty, Pusa delicious, Ranchi (variety from Bihar, popular in South India), CO1, CO2, CO3, IIHR39, IIHR54 (developed at IIHR, Bangalore) and Washington cultivated for fruit and papain production purposes. Coorg honey dew, Coorg green, Pusa delicious and Pusa nanha are commonly grown in Karnataka and Kerala. It has remarkable antioxidant properties in all its parts like the leaf, fruit and seeds. The present study was undertaken to evaluate the leaf extract of *C. papaya* (Linn.) in modulating the radiation induced hematological and tissue damages in Swiss albino mice.

Materials and Methods

Ethical approval:

The study was ethically approved by the Institutional Animal Ethics Committee of the K S Hegde Medical Academy, Nitte University Ref. KSHEMA/IAEC/17/2013 dated 16.12.2013

Chemicals:

Commercially available diallyl disulphide was purchased from TCI chemicals pvt. Ltd., Japan. The other chemicals were purchased from CDH chemicals, Mumbai.

In-vivo study:

Male Swiss albino mice, 4-6 weeks old with 30±5g body weight were selected for the study. They were maintained in the institute's animal house and were provided with food and water *ad-libitum.* They were divided into 6 groups containing 6 mice in each group.

Drug Administration:

The aqueous extract of *Carica papaya* (L.) was chosen for the study as it was soluble and suitable for oral administration. The extract was fed orally in 3 different doses for 15 consecutive days. The change in body weight was noted. Doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight was chosen for the study based on the toxicity studies (Halim SZ et al., 2011). A volume of 0.1mL/10g of *Carica papaya* (L.) aqueous leaf extract was administered using oral gavages.

Irradiation of mice:

The irradiation work was carried out at the Microtron center, Mangalore University. On the 15th day, one hour after the administration of extract, the mice were placed in well- ventilated perspex boxes with a dimension 3 X 5cms. They were irradiated with a sub-lethal dose of 6Gy of electron beam radiation at a dose rate of 72Gy/min, with a source to target distance of 30cms (Ganesh Sanjeev 2012).

Dissection of mice:

The mice were dissected after 24 hours of irradiation. The whole blood was collected by cardiac puncture for hematological estimations. The organs like liver, kidney, brain and spleen were dissected and weighed. The bone marrow was removed for cytological studies.

Hematological Studies:

The hematological studies were done using Erma veterinary blood cell counter (PCE-210VET) using the whole blood collected in 2% EDTA.

Storage of Samples (Nina Holland T et al., 2003):

The whole blood was collected in 2% EDTA tubes and processed for hematological studies within 3 hours of collection. The serum/ plasma were separated and stored in Panasonic (MDF-U334-PE) biomedical freezer at-300C until further processing.

Splenic Index (Damodhara Gowda KM et al., 2013):

The spleen dissected out from the mice was weighed and the splenic index was calculated using the formula:

Splenic Index = Weight of spleen ×100/Body weight

Antioxidant Studies:

Suitable spectrophotometric methods were used and the measurements were recorded in Systronics PC based double beam UV spectrophotometer 2202.

Biochemical Estimations:

The biochemical parameters like urea, creatinine, bilirubin, serum glutamate oxalocetate transaminase (SGOT), serum glutamate pyruvate transaminase, total protein and albumin were estimated using the commercially available kits from Beacon diagnostics, Mumbai, India pvt. ltd.

Total Antioxidant Capacity (Prieto P et al., 1999):

In this assay, 100µL of sample was treated with 100 µL of tri-chloroacetic acid. The mixture was allowed to stand for 5 minutes and centrifuged at 3000 rpm. 100µL of supernatant was taken and 1ml of molybdic acid reagent was added. The mixture was kept in boiling waterbath for 90 minutes. The absorbance was read at 695nm. The molybdic acid reagent contained 0.6M sulphuric acid, 28mM sodium dihydrogen ortho-phosphate and 4mM ammonium heptamolybdate.

Lipid Peroxidation and Membrane Stabilization:

Formation of Malondialdehyde (Okhawa H et al., 1979):

The formation of malondialdehyde was estimated by diluting 0.1mL of sample with 0.4mL of distilled water and 1mL of trichloroacetic acid – thiobarbituric acid reagent (TCA-TBA reagent). The reaction mixture was kept in a boiling waterbath for 15minutes. The endpoint was measured at 535nm and the concentration of malondialdehyde calculated using standard curve.

Reduced Glutathione (Moron MA et al., 1979):

The reduced glutathione was estimated by treating 0.1mL of sample with 1.5mL of precipitating solution containing meta-phosphoric acid sticks and sodium chloride. The mixture is allowed to stand for 10 minutes and centrifuged. 0.5mL of this supernatant was treated with 2mL of 0.3M phosphate solution

and 0.25mL of 5, 5'- dithio bis (2- nitrobenzoic acid). The absorbance was read at 412nm within 10 minutes and calculated using a GSH standard curve.

Evaluation of cytotoxicity (Michael Fenech, 2000)

The cytotoxic effects of the drug was evaluated in bone marrow cells. The bone marrow was flushed with 5% bovine serum albumin (BSA) and smeared on a clean glass slide. The smear was then fixed in methanol, stained with May-Grunwald stain and Giemsa stain. The slide was then dried and observed for polychromatic erythrocytes (PCE) and norchmochromic erythrocytes (NCE) with or without micronucleus and expressed in percentage.

Determination of IL-6:

Interleukin- 6 (IL-6) belongs to cytokine family and is a hematopoietic factor which plays an important role in cell proliferation & immunomodulation. IL-6 levels in mice serum was estimated by ELISA method and the readings were recorded in an automated ELISA reader using Biolegend ELISA Kit, San Diego.

Statistical Analysis:

The results are expressed as mean ± standard deviation. Analysis of data and comparison was done using one way ANOVA with Tukey's multiple comparison test using PRISM 3.0 software. The p value less than 0.05 was considered significant.

Results and Discussion

Hematological studies:

Figure 1 shows the changes in hemoglobin, RBC and packed cell volumes in control, radiation control, and 3 doses of papaya extract treatment prior irradiation groups. There is a significant decrease ($p<0.05$) in Hb levels, RBC levels and PCV in radiation control when compared to the control (non-irradiated) group. The papaya extract treatment prior to irradiation retained the levels of Hb, RBC and PCV when compared to control groups indicating the protective effect of the extract. There is a significant decrease ($p<0.01$) in the WBC levels of radiation control groups when compared to control and in the extract treated groups the values remain reduced when compared to control but at a slightly higher range when compared to the radiation control (table 1). There is no significant change in the differential count in the irradiated and non-irradiated groups (table 1). There is a significant decrease ($p<0.05$) in the platelet count of the radiation control group when compared to control and the papaya treatment prior irradiation retained platelet count in the normal range.

Biochemical Studies:

Among the biochemical parameters there is a significant increase ($p<0.05$) in the serum urea levels of radiation control and the papaya extract treatment prior irradiation groups show a dose dependant decrease in the serum urea levels (table-2). In the serum creatinine levels there is no significant changes were seen. There is a significant increase ($p<0.05$) in SGPT levels of radiation control group and the papaya extract treatment the values are reduced compared to the radiation control indicating restoration of liver function (table 2). There is no significant change seen in the bilirubin, SGOT, ALP, total protein and albumin levels (table 2). There is also a significant decrease in the total antioxidant capacity of radiation control when compared to control group and there is no significant change seen in the papaya extract treatment prior irradiation groups (table 3).

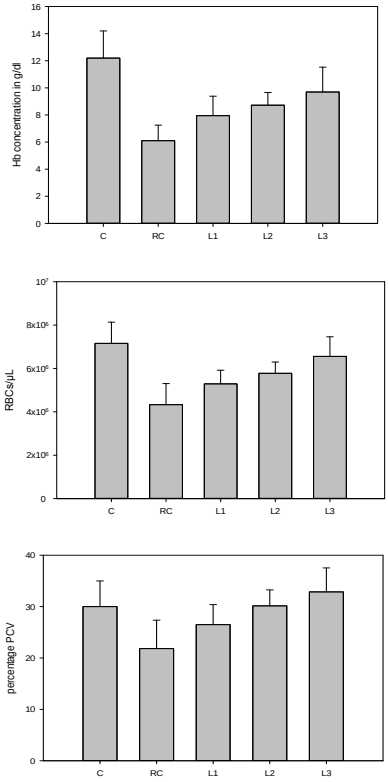


Figure 1 showing the hemoglobin, RBC & hematocrit values obtained in control(C), radiation control (RC), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3). The hemoglobin levels are expressed in g% and RBC count in cells per microlitre of blood and packed cell volume in %.

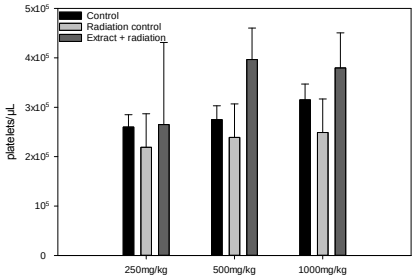


Figure 2 showing the platelet count obtained in control, radiation control, and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3 respectively). The platelet values are expressed in platelets / microlitre of blood.

Lipid peroxidation and membrane stabilization:

There is a significant increase ($p<0.05$) in the malondialdehyde formation in the radiation control group when compared to the control group and there is no significant change in the papaya extract treatment prior irradiation groups (figure 3). GSH levels were decreased ($p<0.05$) in the radiation control group

Table 1 showing the total WBC count, & differential count obtained in control(C), radiation control (RC), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3). The WBC levels are expressed in cells/microlitre of blood and the differential count in percentage number of cells.

Parameters Groups	Total WBC count cells /µL	% Granulocytes	% Lymphocytes	% Monocytes
Control	4126 ± 200	11 ± 3	88 ± 15	1 ± 1
Radiation Control	1467 ± 602.77**	14.03 ± 6.44	79.87 ± 7.84	6.1 ± 1.73
Papaya Leaf Extract (250mg/kg)	1875 ± 906.91*	7.425 ± 4.28	86.3 ± 6.57	6.275 ± 2.41
Papaya Leaf Extract (500mg/kg)	1825 ± 713.55*	7.55 ± 1.2	85.85 ± 4.06	6.6 ± 2.88
Papaya Leaf Extract (1000mg/kg)	1575 ± 1268.52*	9.375 ± 5.12	86.05 ± 4.59	4.575 ± 1.8

The values are expressed as mean ± SD. *p value<0.05, **p value <0.01, ***p value <0.001

Table 2 showing the Biochemical Analyses done in control (C), radiation control (RC), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3).

Parameter	Control	Radiation Control	CP Leaf (250mg/kg)	CP Leaf (500mg/kg)	CP Leaf (1000mg/kg)
Urea (mg/dl)	5.4 ±1.2	102 ± 10.2**	53±5.3*	35±3.5*	9±1.1**
Creatinine (mg/dl)	0.3±0.2	0.6±0.3	0.7±0.4	0.6±0.3	0.7±0.3
Bilirubin (mg/dl)	0.8±0.2	0.9±0.3	0.8±0.4	0.7±0.3	0.7±0.4
Total Protein (g/dl)	9±1.2	8±1.1	8±0.8	6.2±1.1	7.6±1.4
Albumin (g/dl)	3.8±1.2	3.6±1.4	3.4±1.5	3.5±1.4	3.6±1.5
SGOT (U/L)	160±22	670±78.5	149±58.6	540±83.2	820±79.4
SGPT (U/L)	44±4.4	330±33**	226±22.6*	130±13*	250±25*
ALP (U/L)	50±26.3	80±35.4	192±37.8	90±45.3	240±36.4

The values are expressed as mean ± SD. *p value<0.05, **p value <0.01, ***p value <0.001

Table 3 showing the blood levels of total antioxidant capacity in control (C), radiation control (RC), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight respectively)

Parameter	Control	Radiation Control	CP Leaf (250mg/kg)	CP Leaf (500mg/kg)	CP Leaf (1000mg/kg)
Total Antioxidant Capacity (Ascorbic Acid equivalents)	0.062±0.025	0.0007±0.001	0	0	0.015±0.001**

The values are expressed as mean ± SD. *p value<0.05, **p value <0.01, ***p value <0.001

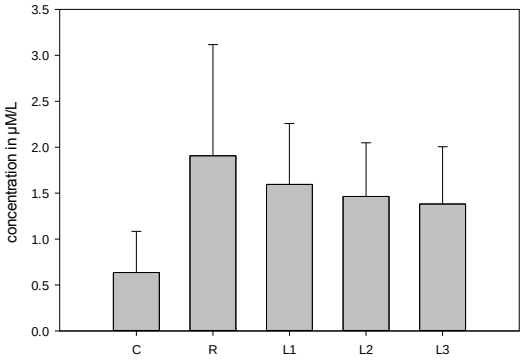


Figure 3 showing the levels of malondialdehyde in control (C), radiated (R), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3 respectively).

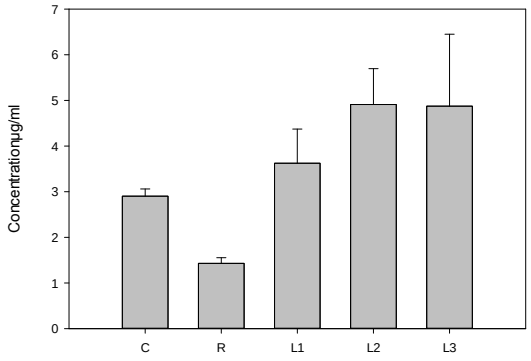


Figure 4 showing the levels of reduced glutathione in control (C), radiated (R), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3 respectively).

Splenic Index:

when compared to the control group and in the papaya extract treatment prior irradiation groups GSH levels remain at a higher level compared to the radiation control (Figure 4). Splenic index depends on the body weight of mice and also on the spleen weight. The body weight of mice was reduced in the radiation control and also decreased spleen weight reduced the splenic index in the radiation control compared to control group. Papaya extract treatment prior irradiation has controlled the splenic index in comparison with the radiation control (figure 5).

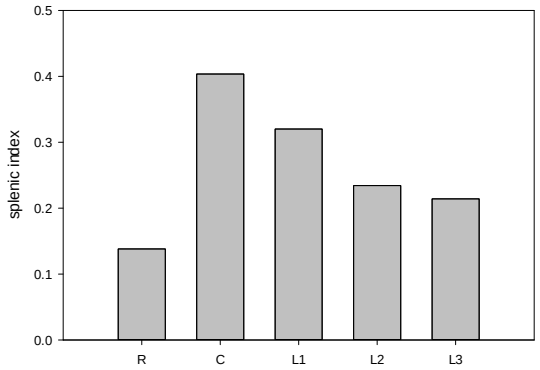


Figure 5 showing the splenic index seen in control (C), radiated (R), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3)

Evaluation of cell proliferation and immunomodulation by the estimation of interleukin-6:

Interleukin- 6 (IL-6) belongs to cytokine family and is a hematopoietic factor which plays an important role in cell proliferation and immunomodulation. There is significant increase ($p<0.01$) in the IL-6 expression at a dose of 1000mg/ml aqueous leaf extract of *Carica papaya* (Linn.) (figure 6).

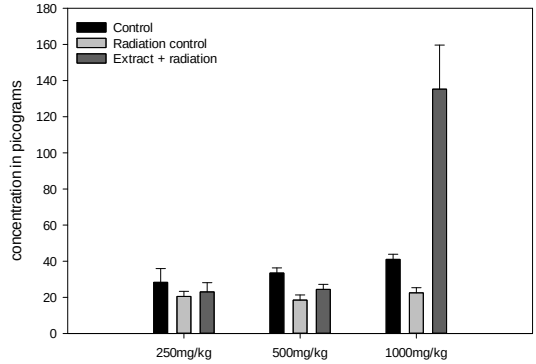
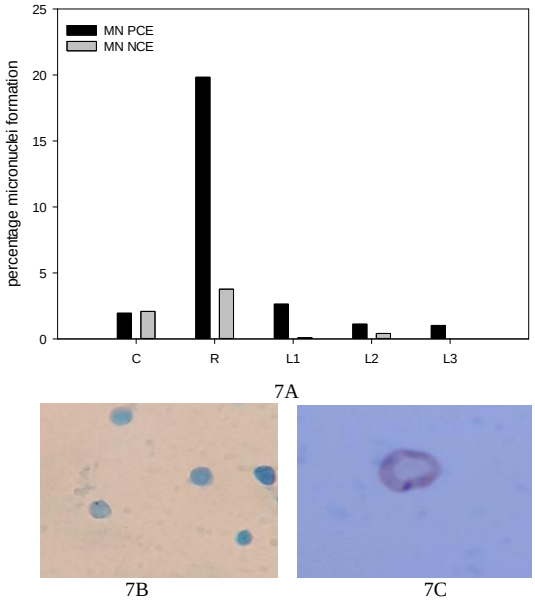


Figure 6 showing the IL-6 levels in control, radiation control and extract + radiation groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight)

Evaluation of cytotoxicity by micronucleus assay:

There is an increase ($p<0.05$) in the number of MnPCE and MnNCE in radiation control group compared to the control group. In the papaya extract treatment group prior irradiation there in reduction in the number of both MnPCE and MnNCE (figure 7A, B, C).



7A

7B 7C

Figure 7A showing the graphical representation of micronucleus formation seen in control (C), radiated (R), and extract + radiated groups with 3 different doses of *C. papaya* (Linn.) aqueous leaf extract (250mg, 500mg and 1000mg/kg body weight: L1, L2, L3) 7B showing micronucleated polychromatic erythrocyte and 7C showing micronucleated normochromatic erythrocytes

Radiation has shown to induce various biological effects in mice. Studies have shown that radiation reduces the hematological parameters like hemoglobin, red blood cell count, white blood cell count, platelet count and inhibits hematopoiesis (Preeti Verma et al., 2011). Radiation has shown to induce oxidative stress by increasing membrane peroxidation and reducing antioxidants in the blood. There is an overall reduction in the blood antioxidants, reduced glutathione and the antioxidant enzymes like superoxide dismutase, catalase, glutathione S transferase and glutathione peroxidase (Naziya Begum et al., 2012). All these enzymes play a very important role in reducing the radiation induced oxidative stress. Radiation has also been shown to induce formation of micronucleus in the bone marrow and peripheral blood cells indicating cytogenetic damage (Jagetia GC et al., 2002). Radiation also afflicts damages to the liver and kidney thereby reducing their normal function. (Goksel Sener et al., 2006)

Previous studies have shown the efficacy of papaya extracts as nephroprotective (Olagunju et al., 2009), tissue protective (Josiah et al., 2011), hepatoprotective (Kingsley, 2012) and as platelet enhancer (Kala, 2012). The mice were whole body exposed to 6Gy sublethal dose of electron beam radiation. There was a significant reduction in the hematological parameters. The RBC counts were reduced in the irradiated groups compared to the control group ($p<0.01$). There was a

drastic reduction in the WBC levels of the irradiated groups compared to the control groups ($p<0.001$). Further there is significant reduction in the hematocrit values ($p<0.001$) and platelet levels compared to the control groups ($p<0.01$). There was an increase in the malondialdehyde levels of irradiated groups and decrease in the glutathione (GSH) levels ($p<0.05$) indicating lipid peroxidation. There is also a decrease in the total antioxidant levels and antioxidant enzyme levels but they are only clinically reduced in the irradiated groups compared to control groups but is not statistically significant. At 6Gy electron beam radiation, there is approximately 20% increase seen in the number of polychromatic erythrocytes present in the bone marrow of irradiated groups compared to the control group indicating enhanced cytogenetic damages.

Previous studies done by EV Ikpeme et al. (2011) have demonstrated the hematological potential of *C. papaya* (L.) leaf extracts. The median lethal dose for *C. papaya* (Linn.) was found to be >2500 mg/kg body weight in rats (Oduola T et al. 2007). In the present study also papaya leaf extract (pre-treatment + irradiation) has shown enhanced hemoglobin and hematocrit levels compared to the radiated groups ($p<0.05$). There is marginal enhancement in the WBC counts when compared to the radiation control groups. There is a significant increase in the platelet levels ($p<0.01$) in the low doses of papaya leaf extract treatment. Recent studies have concluded that *C. papaya* leaf extract significantly increased the platelet and RBC counts in murine models (Chandi Asoka SL et al., 2013). According to a study by Swathi Patil et al., (2013) *C. papaya* leaves contain various phytoconstituents like saponins, tannins, cardiac glycosides and alkaloids. The alkaloids present include carpaine, pseudocarpaine and dehydrocarpaine I and II. These constituents can act on the bone marrow, prevent its destruction and enhance its ability to produce platelets. Moreover, it can also prevent platelet destruction in the blood and thereby increase the life of the platelet in circulation. There is marked improvement in the splenic index in papaya extract (pre-treatment + irradiation) groups compared to the radiation control. This partly demonstrates the reduced hematopoietic stress on the secondary hematopoietic organ, the spleen. Further, it has enhanced the Interleukin-6 levels many folds compared to the irradiated groups. IL-6 has been known to be a pivotal cytokine in the body's defenses through the induction of immune response as well as in the stimulation of hematopoiesis (Francis Herodin et al., 1992). Aqueous extract of *C. papaya* leaves has shown anti-tumor activity and immunomodulatory effects (Noriko Otsuki et al., 2010). Although there is no significant change in the antioxidant enzyme levels there is stabilization in the reduced glutathione levels in the groups treated with the extract prior to irradiation indicating reduced lipid peroxidation and enhanced membrane stabilization. The liver and kidney function is not affected but there is an increased urea levels in the serum of irradiated group and subsequent reduction in the papaya extract treated groups indicating possible reduction in the protein degradation. The papaya extract (pre-treatment + radiation) treated groups have shown reduction in the number of micronucleus in the polychromatic and normochromatic erythrocytes. The present study has shown improvements in Hb, RBC, platelet count at 1000mg/kg (bd. Wt.) concentrations compared to the radiated groups. Reduction in radiation induced hematopoietic stress, formation of micronucleus, biochemical changes have been shown at 1000mg/kg

Conclusions

The present study attempts to determine the potential of *C. papaya* (Linn.) as a natural source of radiation protection. It is clear from the present study that aqueous leaf extract of *C. papaya* (Linn.) has hematopoietic and tissue protective potential. As we have an enormous variety of flora, there is significant possibility of developing an efficient radioprotector from these natural resources. *C. papaya* (Linn.) promises to be a novel hematopoietic enhancer in radiotherapy of cancer and also as an ideal 'radio-protector' to counter radiation induced damages in radiation emergencies. Further studies can be undertaken to identify and isolate the compounds involved to provide maximum protection and determine the exact mode of action in radiation protection.

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