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PROTECTION TO RADIATIONAL HAEMATOLOGICAL CHANGES BY CURCUMA LONGA (L.) RHIZOME EXTRACT IN SWISS ALBINO MICE

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ABSTRACT

Gamma radiation (5 Gy) causes a decrease in the number of erythrocyte and leucocyte counts, hemoglobin content and hematocrit percentage, MCV and MCH was scored in Swiss albino mice. In preferred with *Curcuma longa* mice a recovery pattern in haematological parameters was seen after initial radiational changes and normal values were regained by day 30. Present study suggests that use of *Curcuma longa* (L.) extract provide protection against radiation-induced haematological alterations in Swiss albino mice. This investigation may be helpful in formulating herbal medicines against radiation induced damage.

INTRODUCTION

The problem of radiation hazard to living being has risen due to natural background radiations, increasing use of nuclear energy in industry, occupational and medical field such as radiotherapy and face a major drawback because it produces severe side effects developed due to damage to normal tissue. Free radicals are generated by radiation energy in the cells and their reactions with DNA, RNA, and organelle cause cell dysfunction, mortality, mutagenesis or carcinogenesis (Pradhan *et al*., 1973). Evidently, rapidly dividing cells like epithelial and haemopoietic system are prone to early and marked damage to chromosomes as well as other organelle due to higher content of oxygen and water with a higher level of free radical generation on impact of radiation energy (Adhvaryu *et al*., 2008). Radioprotectors are administered to patients to reduce the toxic, mutagenic and carcinogenic effects of ionizing radiation on normal tissues. In these cases phytochemicals are of particular interest due to their antiemetic, anti-inflammatory, antimicrobial, antioxidant, hematopoietic, immunostimulant, metal chelating, and wound healing activities (Weiss and Landauer, 2003). The use of plants and natural products which acts as antioxidants may be beneficial in protecting against the radiation-induced damage, as they are less toxic or non-toxic compared to the synthetic compounds.

Curcuma longa belonging to family Zingiberaceae, grows widely all over India, is a perennial herb of 2-3 ft hight with short stem and short and thick rhizomes. The active ingredients are tetrahydrocurcuminoids (Osawa *et al*., 1995), curcumin, demethoxycurcumin and bisdemethoxycutcumin. Plant extracts were found to have a wide spectrum of therapeutic effects such as anticholesterol activity, fibrinolytic action, anti inflammatory (Ammon *et al*., 1993), antioxidative (Osawa *et al*., 1995), antitumor, imunomodulatory (Antony *et al*., 1999), antimicrobial and hepatoprotective. No acute toxicity in mice was observed on administration of turmeric powder with dose as high as 10g/kg-bw (Sittisomwong, *et al.,* 1990). Natural products of plant origin may prove to be protective against ionizing radiations if they counter the harmful effects of radiation induced free radicals. Evidences support that *Cucuma longa* rhizome is one of the popular antioxidants used for various ailments. The present study has been undertaken to evaluate the effect of aqueous extract of rhizome of *Curcuma longa* (L) on Gamma radiation induced hematological alterations in Swiss albino mice.

MATERIALS AND METHODS

Experimental animal

Twenty four Swiss albino mice (25-30 g) reared in the animal house of Mahavir Cancer Sansthan, Patna, randomly divided into four groups, were kept in cages in a temperature 24 $\pm$ 1ºC, humidity 55 $\pm$ 5%, and lighting 12-h light/dark cycle controlled room. Food and tap water were given *ad libitum* throughout the study. All animal experiments were carried out as per CPCSEA guidelines (Approval No.-1129/bc/07/CPCSEA).

Source of irradiation

Anaesthetized animals were exposed to gamma radiation (Co-

60) at a distance (SSD) of 80 cm from the source to deliver the 5 Gy radiations at the dose-rate of 217.1c Gy min^{-1} in field size of 16×24 cm, at the Radiotherapy Department of M.C.S, Patna.

Preparation of rhizome powder and aqueous extract

Rhizome of *Curcuma longa* plants were collected from the campus of B.M.D College campus and rhizome were shade dried, powdered by grinder. Mixed powders in distilled water and different concentrations were used for the experiment.

Experimental protocol

Animals were divided into different groups, containing six mice each. After acclimatization, animals were treated as follows: the control group (group-I) received tap water, while the experimental groups received orally, the different concentration dose at 500mg/kg to 3000mg/kg b.wt of aqueous extract of *C. longa*. The treatment volume of aqueous extract was determined based on body weight. The toxicological effects were observed in terms of mortality expressed as LD_{50}. No mortality was recorded during the experimental period. Acute oral LD_{50} of the aqueous extract of rhizome of *Curcuma longa* was calculated by using software for probit analysis (EPA probit analysis program, used for calculating LC/EC value, Version 1.5) as determined earlier of M.C.S., Patna was taken in account. Visible physical abnormalities or abnormal demeanor of the mice was recorded during the experimental period.

These animals were observed daily for any sign of sickness, morbidity, behavioral toxicity and mortality. Necropsy was done on 6 animals from each group after week 1, 2 and 4 post-treatment intervals.

Hematological study

Blood samples were obtained by orbital sinus puncture of mice of different experimental groups in a vial containing 0.5 M EDTA for hematological analysis. The haematological parameters of the blood samples were then estimated by standard procedures using Cell Counter (Medonic M- Series, Department. of Pathology, Mahavir Cancer Sansthan, Patna). The haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and white blood cell count (WBC) were determined.

Statistical analysis

Each experimental value was expressed as the mean ± SEM and P value was calculated using one way analysis of variance (ANOVA). $p<0.05$ was considered statistically significant.

Experimental mice were divided into 4 groups

Group	Treatment
I	Unirradiated mice received food and distilled water (DW) only
II	Unirradiated mice was treated orally with food and queous extract of rhizome of *Curcuma longa* at a dose of 200mg/ Kg b.wt./day for 15 consecutive days.
III	Mice received distilled water and then exposed to single doseof 5Gy of gamma- radiation (Co-60).
IV	Mice was treated orally with food and aqueous extract of *Curcuma longa* at a dose of 200mg/Kg b.wt./day for 15 consecutive days. On the 15th day after one hour of administration of dose of aqueous extract exposed to single dose of 5 Gy Gamma-radiations.

RESULTS

The experimental groups (group-I and II) have not shown any noticeable signs of behavioral changes, sickness and mortality. Pretreatment of mice with aqueous extract of *Curcuma longa* (group-II) did not show adverse effects on hematological parameters and levels of RBC, MCV, HGB and MCH were statistically not significant ($p > 0.05$) when compared to control group-I. The present study revealed that after exposure to 5 Gy gamma radiations the haematological parameters (RBC, MCV, PLT, WBC and HGB) exhibited alterations and decrease in the number of erythrocyte and leucocyte counts, hemoglobin content, hematocrit percentage, MCV and MCH was scored in the experimental groups III and IV. In irradiated mice (group-III), a marked decrease was recorded in all hematological parameters when compared to control group-I, whereas group-IV which was pretreated with aqueous extract of *C. longa* then exposed to gamma radiations, the alterations in hematological parameters were less severe. Thereafter, a recovery was recorded in experimental animals which were more pronounced in pretreated group-IV. However, the normal values could not be obtained even after 4 weeks in experimental group-III and a near normal value of hematological parameters were regained by 4 week post-treatment period in group-IV as shown in Table 1. Levels of RBC, MCV, HGB and MCH were statistically very significant ($p < 0.01$) for groups III and IV when compared to control group-I (Figs. 1, 2, 5 and 6). The levels of WBC and HCT for all the experimental groups were very significant ($p < 0.01$) when compared to control group-I (Table 1 and Fig. 3 and 4).

DISCUSSION

In mice, death is due to the

Table 1: Haematological study on the mice post whole body Gamma irradiation and treatment by aqueous extract of rhizome of *curcuma longa*

Haematological parameter	Group-I	Group-I1	After 1 week Group-III	After 2 week Group-IV	After 4 week Group-III	Group-IV	Group-III	Group-IV
RBC (10^6/mm^3)	8.65±0.045	8.80±0.060	6.25*±0.065	7.11*±0.059	7.14*±0.053	8.03*±0.068	7.54*±0.062	8.14*±0.054
MCV(fl)	44.5±0.11	45.05±0.048	42.87*±0.062	44.08*±0.090	43.84*±0.111	44.88*±0.040	44.0*±0.059	45.96*±0.078
HCT (%)	38.35±0.059	39.91*±0.067	26.98*±0.049	30.72*±0.070	34.57*±0.093	36.11*±0.066	35.0*±0.061	36.84*±0.54
WBC(10^3/mm^3)	18.91±0.039	20.77*±0.096	2.61*±0.086	5.71*±0.071	4.63*±0.062	7.41*±0.068	5.0*±0.84	16.0*±0.62
HGB(gm/dl)	15.75±0.056	15.80±0.055	10.77*±0.086	11.97*±0.097	14.49*±0.051	14.79*±0.060	14.9*±0.53	15.2*±0.45
MCH(pg)	18.01±0.082	17.95±0.085	16.20*±0.119	17.04*±0.843	15.88*±0.080	17.57*±0.065	17.9*±0.76	19.2*±0.068

All values expressed as mean ± SEM;*Significant differences at $p < 0.05$

hemopoietic damage inflicted by radiation to the hemopoietic organs like the bone marrow from 11 to 30 days (Jagetia *et al*., 2003). It has been reported that a significant decrease in the hematological constituents of peripheral blood in animals of the irradiation alone group was observed. The decline in hematological constituents may be attributed to a direct damage by radiation. The whole body irradiation of the moderate dose range (5-10 Gy) leads to a decreased concentration of all the cellular elements in the blood. This may be due to a direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and loss of production of cells (Casarett, 1968). Mitotically active precursor cells are sterilized by radiation, and the subsequent supply of RBCs, WBCs and platelets is thereby diminished. The time at which the number of circulating cells in the blood reaches minimum value since, mature circulating cells begins to die off and the supply of new cells from the depleted precursor population is inadequate to replace them so that the full effect of radiation becomes apparent (Hall, 2000).

There is decrease in RBC count and haemoglobin content in irradiated groups, however, the RBC count was significantly higher in experimental group-IV than in group-III. The decrease in haemoglobin content is attributed to the decline in the number of red blood cells and may also be due to the depletion in the synthesis of hemoglobin after radiation exposure. Recently, Samarth and Kumar (2003) observed a similar protective efficacy of plant extract of *Mentha piperita* against radiation-induced depletion in RBC and hemoglobin. In *C. longa* pre-treated animals haemoglobin values were higher at all radiation doses, which indicate a significant protection of erythrocytes by *C. longa*. An increase in erythropoietin level by *C. longa* is also directly responsible for an increase in haemoglobin content.

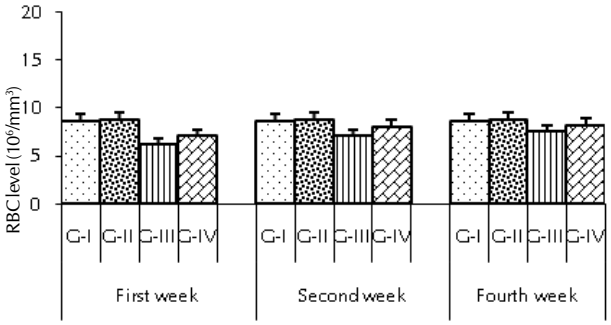

Figure 1: Variations in RBC level in experimental groups of mice

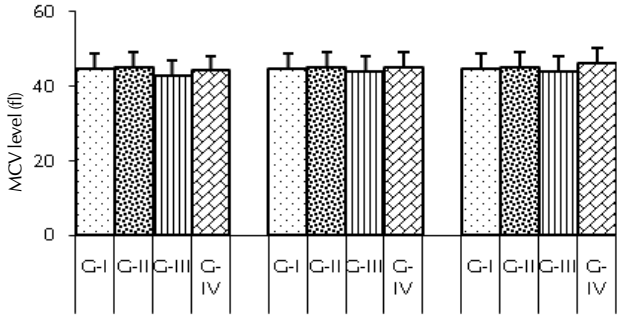

Figure 2: Variations in MCV level in experimental groups of mice

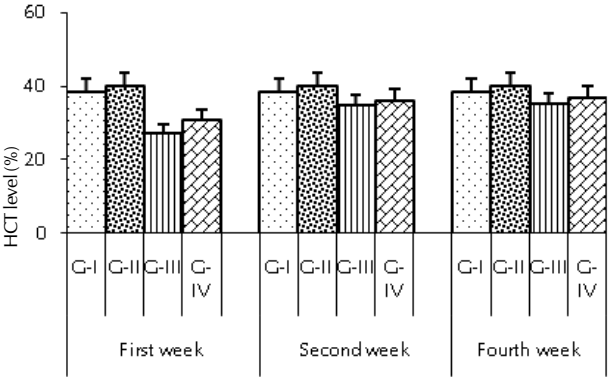

Figure 3: Variations in HCT level in experimental groups of mice

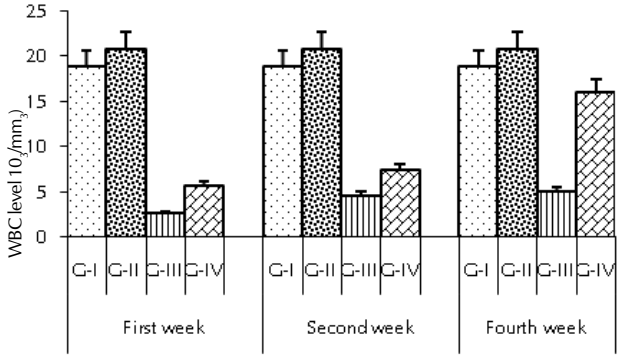

Figure 4: Variations in WBC level in experimental groups of mice

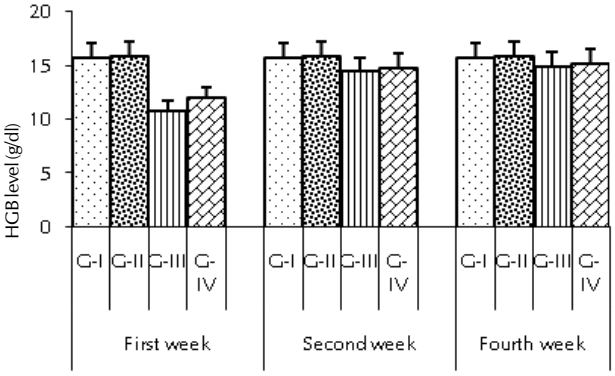

Figure 5: Variations in HGB level in experimental groups of mice

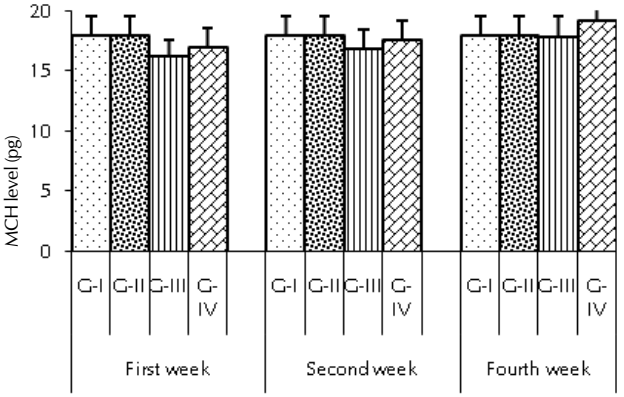

Figure 6: Variations in MCH level in experimental groups of mice

MCV and MCH decrease due to decrease in size of RBC, destruction of number of RBC or impaired biosynthesis of heme in bone marrow (Ismail and Aziz, 2000).

In the present investigation, a drastic reduction in leucocytes count after irradiation is in agreement with the findings of earlier workers (Baum *et al*., 1969; Goldin and Neff, 1975). In *C. longa* pretreated animal groups, the total leucocyte count and lymphocyte percentage were higher than the control group. A similar protection in lymphocyte count has been observed while using cysteine (Patt *et al*., 1957) and MPG (Kumar and Umadevi, 1983) in mice prior to irradiation.

Haematocrit (HCT or PCV) is the percentage of whole blood that is made up of cells and a decrease to below normal in its value indicates anaemia. The decline in haematocrit values is due to decreased erythropoiesis and increased plasma volume. The decrease in the number of erythrocytes in the present study also supports the view of decreased erythropoiesis as the cause of a decline in haematocrit. In *C. longa* treated animals, hematocrit values showed a consistent recovery over control.

Several pathways of radioprotection have been suggested for the mechanism of protective action in mammalian cells against the damaging effects of ionizing radiation. The mechanisms implicated in the protection of cells by radio protectors include free radical scavenging that protects against reactive oxygen species (ROS) generated by ionizing radiation or chemotherapeutic agents, and hydrogen atom donation to facilitate direct chemical repair at sites of DNA damage. The ROS generated by ionizing radiation are scavenged by radioprotectors before they can interact with biochemical molecules, thus reducing the harmful effects of radiation. The active ingredient is Curcumin, essential oil (p-tolymethylcarbinol), present in Rhizome of the plant (Encyclopedia of Natural Medicine, 1998) and Curcumin is a antioxidant and also a good scavenger of reactive oxygen species and lowers its formation as well as the formation of inflammatory compounds such as prostaglandins and leukotrienes (Unnikrishnan and Rao, 1992) and protects erythrocyte membranes and phospholipid fatty acids from oxidation (Leela *et al*., 1992). It is also attributed to stimulating or protecting hematopoiesis in bone marrow and the subsequent increase of hematological constituents in the peripheral blood so it acts as a radio protector.

The results of the present study indicate that pretreatment with aqueous rhizome extract of *C. longa* protected Swiss albino mice from radiation induced hematological alterations. Hematological counts serve as sensitive parameters determining the protective efficacy of any compound (Garima and Goyal, 2008). The radio protective effect of *C. longa* was demonstrated by evaluating the hematological parameter such as Hb, RBC count, WBC count, HCT (hematocrit), MCV and MCH after various post irradiation time intervals *i.e.,* from week 1 to 4. A significant radioprotection was achieved when *C. longa* was given orally (200 mg/kg body weight/day) for fifteen consecutive days before radiation exposure (5 Gy gamma radiation).

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