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Radioprotective effect of *Eclipta alba* (L.) against radiation induced haematological changes in Swiss albino mice

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ABSTRACT

Eclipta alba has diverse pharmacological and antioxidative properties which aroused interest to obtain insight into the radioprotective effect of its aqueous leaf extract against gamma radiation induced haematological alterations in Swiss albino mice. Mice were treated for 15 days with a dose of 250mg/kg b.wt of *E. alba* (L.) aqueous leaf extract selected on the basis of LD₅₀ (2413.407mg/kg b. wt) and then exposed to single dose of 5Gy gamma radiation. Exposure to single dose of 5Gy gamma radiation in untreated mice (group-III) resulted in highly significant decrease in levels ($P<0.01$) of hematological parameters of RBC, MCV, HGB and MCH and the decrease was statistically very highly significant ($P<0.001$) for WBC and HCT as compared to control group-I. Exposure to gamma radiations in *E. alba* pre-treated group-IV mice showed less severe alterations in haematological parameters. However, the normal values could not be obtained after two weeks in group-III while near normal values of haematological parameters of RBC, MCV, HGB and MCH except WBC were regained after two weeks in treated group-IV. Preliminary phytochemical analysis of aqueous leaf extract of *E. alba* showed positive tests for alkaloid, phytosterols, triterpenoid and flavanoids, saponins, tannins, sugar, gum and mucilage. Thus *E. alba* aqueous extract modulates radiation induced hematological alterations which may be probably due to its antioxidative activity.

Keywords: Radiation; *Eclipta alba*; Mice; Antioxidant; Haematology.

INTRODUCTION

Natural products may be beneficial in protecting against the radiation-induced damage, as they are less toxic or practically non-toxic compared to the synthetic compounds at their optimum protective dose levels (Muktika, et al., 2007). The exposure of animals to a single whole-body dose of ionizing radiation results in a complex set of symptoms whose onset, nature and severity are a function of both total radiation dose and radiation quality and it is mainly classified into three syndromes

haematopoietic syndrome, the gastrointestinal syndrome and the central nervous system syndrome (Arena, 1971). According to Broerse and Mac (1984), Haemopoietic syndrome occurs at the lowest radiation doses (<10 Gy) and is manifested by haemopoietic stem cell depletion and ultimately by depletion of mature haemopoietic and immune cells.

Therefore, the interest has been increased in development of potential drug of plant origin for the modification of radiation induced haematological effects. Recent studies have indicated that some commonly used medicinal plants may be good sources of potent but non-toxic radioprotectors such as *Panax ginseng* (Verma, et al., 2010), *Ocimum* (Uma Devi, et al., 2000) and *Mentha* (Samarth and Kumar, 2003), against radiation induced alterations and mortality.

E. alba (Family-Asteraceae), locally known as Kesohraj or Bhringaraja has immunomodulatory (Jayathirrtha and Mishra, 2004), free radical scavenging action (Bhattacharya, et al., 1997), and effective in conditions of anemia (Das, 1992). In various *in vitro* model studies of methanolic extract of *E. alba*, it was found to scavenge superoxide and nitric oxide radicals and has antioxidant properties (Karthikumar, 2007). The antioxidant activity of *E. alba* can be attributed to the presence of phenolic and flavonoids compounds (Unnikrishnan, et al., 2007). Since there are no reported literature on radioprotective effects of antioxidant rich medicinal plant *E. alba* (L.), the present study is an attempt to find out the efficacy of *E. alba* as in modulating the radiation induced haematological alterations in Swiss albino mice.

MATERIALS AND METHOD

Experimental animal: Randomly divided Swiss albino mice weighing 24-30g (4 groups) were housed in stainless-steel wire cages at a temperature of ($24 \pm 1^{\circ}\text{C}$), humidity- ($55 \pm 5\%$), and lighting- (12h light/dark cycle). 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: Un-anesthetized animals were restrained in well-ventilated boxes and were whole-body exposed to gamma radiation (Co-60) at a distance (SSD) of 80 cm from the source to deliver single dose of 5 Gy gamma radiations (field size 16×24 cm) at the dose-rate of 217.1c Gy/min in the Radiotherapy Department of Mahavir Cancer Sansthan and Research Centre, Patna.

Preparation of aqueous leaf extract: *E. alba* (L.) plants was collected from the campus of B.M.D College and identified by The plant was taxonomically identified by Dr. S. Bedi, (Associate Professor, Department of Botany, PWC, Patna University, Patna) and kept in the herbarium of the laboratory under the voucher specimen Number: B.M.D/ BOT/03/10. The leaves were shade dried at room temperature (25°C) for about 10 days, powdered, weighed and mixed in 10-ml of distilled water to obtain the concentrations to be used in the experiment.

Phytochemical investigation of the extract: was conducted by the methods of Harbone (1988) and Kokate (1994).

Acute toxicity study and dose selection: Swiss albino mice were divided into four groups, each containing six mice. After acclimatization, animals were treated as follows: the control group received food and tap water *ad libitum*, while the experimental groups received in addition orally, the different concentrations doses of 500mg/kg, 1750mg/kg, 2000mg/kg, 2500mg/kg and 3000mg/kg b.wt of aqueous leaf extract of *E. alba*, prepared by dissolving 500 mg-3000 mg of dried powder of *E. alba*

leaves in 10 ml of distilled water. The treatment volume of aqueous extract was determined based on body weight. The toxicological effects were observed in terms of mortality expressed as LD₅₀. Based on the experimental observations the acute oral LD₅₀ of the extract was calculated as 2413.407 mg/kg b.wt using software for probit analysis (Environmental Protection Agency PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, Version 1.5) and the treatment dose was thus selected as 250mg/kg/b.wt treatment dose was thus selected as 250mg/kg/b.wt

Experimental design:

Group	Treatment
I	Un-irradiated mice received food and distilled water (DW) only.
II	Un-irradiated mice were treated orally with food and aqueous extract of leaves of <i>E. alba</i> at a dose of 250mg/Kg b.wt./day for 15 consecutive days.
III	Mice received food, DW and then exposed to single dose of 5Gy of gamma-radiation.
IV	Mice were treated orally with food and aqueous extract of <i>E. alba</i> at a dose of 250 mg/Kg.bwt./day for 15 consecutive days. On the 15th day after one hour of administration of dose of aqueous leaf extract, mice were exposed to single dose of 5Gy gamma-radiations.

Animals were observed daily for any sign of sickness, morbidity, and mortality. A minimum of six animals from each group were necropsied on seventh day (1st week) and 2nd week after irradiation in order to evaluate haematological parameters.

Haematological study: Blood samples were collected by orbital sinus puncture from different experimental groups in EDTA vials for hematological analysis. The haematological parameters of the blood samples were then estimated by standard procedures using Cell Counter (Medonic M- Series) in the Department of Hematology, 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), white blood cell count (WBC) and haematocrit percentage (HCT) were thus determined.

Statistical analysis: Each experimental value was expressed as the mean ± SEM and P value was calculated by one way ANOVA. The value was considered statistically significant at P<0.05.

RESULTS

Physical and behavioral changes: Single dose of 5Gy Gamma irradiation on *E. alba* aqueous extract treated group-IV mice did not show toxic effect in terms of sickness, mortality, significant change in body weight, urination and defecation pattern, however, untreated group-III mice showed signs of weakness and lethargy, although general activities of such animals were apparently normal during all 14 days post-irradiation.

Acute toxicity study: Aqueous extract of *E. alba* did not produce any toxic symptoms or mortality upto the dose of 1750mg/kg b.wt. in Swiss albino mice. Based on the experimental observations the acute oral LD₅₀ of the extract was calculated as 2413.407 mg/kg b.wt using software for probit analysis (Environmental Protection Agency PROBIT ANALYSIS PROGRAM, used for calculating LC/EC value, Version 1.5) and the treatment dose selected was 1/10th of LD₅₀ (250mg/kg.b.wt/day).

Phytochemical analysis: Preliminary phytochemical analysis of aqueous leaf extract of *E alba* showed positive tests for alkaloid, phytosterols, triterpenoid and flavonoids (Coumarone), saponins, tannins, sugar, gum and mucilage. It has also been reported by Uddin, (2010) that *E. alba* contains mainly coumestans i.e. wedelolactone and

demethylweddolactone, polypeptides, polyacetylenes, thiophene-derivatives, steroids, triterpenes and flavonoids.

Haematological analysis: The results of haematological changes are shown in table-1. In *E. alba* aqueous extract treated mice (group-II) no significant changes ($P>0.05$) were observed. In untreated single dose of 5Gy gamma irradiated mice (group-III) a highly significant decrease ($P<0.01$) were recorded in haematological parameters of RBC, MCV, HGB and MCH and for HCT and WBC, the decrease was statistically very highly significant ($P<0.001$) as compared to control group-I, whereas *E. alba* aqueous extract treated mice (group-IV) when exposed to single dose of 5Gy gamma radiations showed less severe alterations in haematological parameters except level of WBC count. However, the normal values could not be obtained after two weeks in untreated experimental group-III while a near normal value ($P>0.05$) of haematological parameters RBC, MCV, HCT, HGB and MCH except WBC (Table-1) were regained after two weeks in group-IV pre treated with aqueous leaf extract of *E. alba* for 15 consecutive days.

DISCUSSION

Agents capable of enhancing survival in the radiation dose inducing the haemopoietic syndrome have typically been associated with accelerated haemopoietic regeneration. The decline in haematological constituents may be attributed to a direct damage by radiation. In the present study, radiation causes depletion in the RBC count or erythrocytes (Table-1). According to Fred and Smith (1968), the radiation-induced depletion of haematopoietic stem cells may be an important factor contributing to the decline in the erythrocytic population. *E. alba* pretreated mice (group-IV) showed recovery of normal erythrocytes level in the single dose irradiated mice. In a similar case, *Panax ginseng* treatment caused recovery of erythrocytes count in blood after irradiation (Verma, et al., 2010). There is also noticeable depletion in hemoglobin concentration in Swiss albino mice was found when exposed to 3.6 Gy gamma radiations (Daga, et al., 1995). The decrease in the hemoglobin content may be due to the decrease in the number of red blood cells and/or the leakage of RBC depletion in the synthesis of haemoglobin after radiation exposure. In our study *E. alba* pre-treated mice (Group-IV), the level of haemoglobin was maintained higher as compared to the control values at various intervals, indicating that it may be having a protective action on the hemoglobin content.

The hematocrit (Ht or HCT) or packed cell volume (PCV) is the proportion of blood volume that is occupied by red blood cells. A depression in the hematocrit value can be attributed to total cell depletion in peripheral blood aided by disturbances in steady state mechanisms in blood forming organs as well as an increase in plasma volume after irradiation and is in agreement with the recent findings in the case of treatment with *Emblica officinalis* (Linn.) fruit extract (Singh, 2006). In our study, *E. alba* pretreated mice (group-IV) the HCT value is 36.94% and it indicates that *E. alba* may be providing protection to bone marrow and blood erythropoietic cells and thus maintaining the normal percentage of HCT.

The MCV relates to the average size of the red blood cell. The amount of haemoglobin in a single red blood cell is indicated by the MCH. The level of both MCV and MCH decreased due to decrease in size of RBC, destruction of number of RBC or impaired biosynthesis of heme in bone marrow due to radiation effect (Ismail, 2000). In the present investigation, *E. alba* pretreatment showed a gradual recovery of

haematological constituents in the peripheral blood so it also maintains the level of MCV and MCH of irradiated Swiss albino mice.

In *E. alba* pre-treated animals, leucocytes values were higher at single dose of 5Gy gamma radiation doses, which indicate a significant protection of leucocytes by this plant extract. The initial rapid fall in leukocytes count may be due to a fast decline of lymphocytes in peripheral blood that are the most radiosensitive as revealed by differential leukocyte count. Samarth and kumar (2003), have reported a depression in the number of leucocytes of gamma irradiated mice.

Presence of high quantity of ascorbic acid and phenolic content in *E. alba* that can explain its strong free radical scavenging activity (Uddin, 2010). According to Redpath and Wilson (1973), ascorbic acid reduces radiation-induced sickness and mortality and according to Sarma and Keshavan (1993), protects mice bone marrow cells against radiation-induced chromosome damage. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Rice-Evans, 1997). Since *E. alba* scavenged free radicals like •OH, DPPH and NO• in a concentration dependent manner *in vitro* therefore, free radicals scavenging seems to be one of the important mechanism of radioprotection by *E. alba*. It also contains flavonoids, a class of compounds reported to possess antioxidant and free radical scavenging activities (Korina and Afanasev, 1997; Uddin and Ahmad, 1995).

CONCLUSION

In conclusion, *E. alba* pre-treatment reduced radiation-induced hematological damage. The protection afforded with *E. alba* in the haematological indices in the present study may prove to be beneficial for the clinical use of medicinal plants as radioprotectors.

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Table-1: Hematological alterations in blood of the mice post whole body exposed to 5 Gy gamma irradiation with or without *Eclipta alba* extract treatment.

Hematological parameter	Group-I	Group-II	After 1 week		After 2 week	
			Group-III	Group-IV	Group-III	Group-IV
RBC ($10^6/\text{mm}^3$)	8.67 ± 0.072	8.52 ± 0.079	6.40** ± 0.092	7.47* ± 0.0821	8.11* ± 0.069	8.76 ± 0.103
MCV (fl)	44.1 ± 0.012	43.25 ± 0.029	41.39** ± 0.114	42.7* ± 0.017	42.4* ± 0.022	43.2 ± 0.039
HCT (%)	38.15 ± 0.131	37.05 ± 0.096	26.94*** ± 0.012	31.44** ± 0.058	32.27** ± 0.075	36.94 ± 0.114
WBC ($10^3/\text{mm}^3$)	18.89 ± 0.022	17.21 ± 0.058	2.6*** ± 0.098	4.05*** ± 0.108	5.0*** ± 0.091	7.48** ± 0.113
HGB (gm/dl)	15.8 ± 0.074	14.7 ± 0.088	10.45*** ± 0.107	12.74** ± 0.078	13.88** ± 0.116	14.38 ± 0.095
MCH(pg)	17.95 ± 0.065	17.55 ± 0.103	15.35** ± 0.115	16.65* ± 0.065	17.60* ± 0.016	17.97 ± 0.066

- All values expressed as mean ± SEM.
- *Significant ($P < 0.05$); **Highly Significant ($P < 0.01$); and *** Very Highly Significant ($P < 0.001$)