

RADIOPROTECTION BY ACETONE FRACTION OF CENTELLA ASIATICA ON PERIPHERAL BLOOD CELLS OF MOUSE.

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Summary

Centella asiatica, a well known medicinal plant of India is reported to possess radioprotective activity against lethal dose of gamma radiations. Its aqueous extract was used for the purpose. In the present study its aqueous extract was fractionated and Acetone fraction was used to test its radioprotective activity *in vivo* in mouse. Adult swiss albino mice were divided into two groups and were irradiated with and without *Centella asiatica* pretreatment. The animals were sacrificed at various intervals after treatment. Blood was collected and analysed. It was observed that 25 mg/kg body weight of Acetone fraction of *Centella asiatica* aqueous extract protects the 8 Gy irradiated mouse, when given 1 hr. prior to irradiation. Higher cell counts, higher haemoglobin content and haematocrit values were observed in the plant extract treated animals. It shows that peripheral blood cells are protected by *Centella asiatica*.

Key words : *Centella asiatica*, Radioprotection, Peripheral blood cells.

Introduction

Plants are being tested extensively for their new properties. There are a large number of plants, which were tested for their radioprotective efficacy and many of them were found radioprotective also. *Centella asiatica* is one of them.

Centella asiatica (Linn.) is a small herb, growing in the swamps in various parts of Asia and used around the world for centuries to treat several diseases. In India it is used for various purposes as medicine, as well as a part of food. It is a known general and brain tonic. According to an authentic document prepared by publications and information directorate, Government of India, New Delhi¹ *Centella asiatica* possesses a variety of medicinal properties. *Centella asiatica* is found to improve learning, memory and strengthens central nervous system². Chemical constituents isolated from *Centella asiatica* have shown cytotoxic, antistress, antileprotic, antibacterial, antifilarial, antituberculosis and wound healing capacity. It has been used as an internal and external remedy to various diseases like ulcerations, eczema, leprosy etc. *Centella asiatica* protects against peroxidation reactions, thereby against the cell damage³. It has antilelastase activity and acts as a free radical scavenger also. Methanolic extract of *Centella asiatica* have shown immunopotentiating effect.⁵

Exposure to the gamma radiation causes pathological, hematological and biochemically important changes in the body resulting in metabolic disorders, which in due course of time may lead to cellular damage or death of the cell. Blood is also affected. In the present study Acetone fraction of *Centella asiatica* aqueous extract was tested against Co⁶⁰ gamma radiation in the blood cells of mouse.

Materials and Methods

Animals

The experiments were conducted on Swiss albino mouse, 6-8 week old, weighing 25 gm (± 2 gm), which were selected from an inbred colony and maintained on standard mice feed obtained from Hindustan Lever Ltd., India and water *ad libitum*, Animals were kept in polypropylene cages on paddy husk as bedding, according to WHO guidelines. Departmental Ethical Committee has approved the present study.

Irradiation

The animals were irradiated (Co⁶⁰) with Cobalt-60 Teletherapy unit (ATC-C9) at SMS Medical college and Hospital, Jaipur. The animals were exposed to 8 Gy of gamma radiation whole bodily in a single dose.

Plant Extract

Aqueous extract of whole plant of *Centella asiatica* was obtained from Amsar Private Limited, Indore. Acetone fraction of this extract was prepared by soxhalation and animals were pretreated with this extract orally, at the dose rate of 25 mg/kg body weight, one hour prior to irradiation. The dose of the plant extract was selected on the basis of LD50/30 survival assay.

Experimental Design and Analysis

Animals were divided in the following groups:

- Group I : Animals of this group were sham irradiated
- Group II : Animals of this group received plant extract one hour prior to irradiation at the dose rate of 25 mg/kg body weight, orally, then exposed of 8 Gy of CO⁶⁰ gamma radiation. This group served as experimental group.
- Group II : Animals of this group were irradiated with 8 Gy of CO⁶⁰ gamma rays and given equal amount of double distilled water as given with the plant extract. This group served as control group.

Atleast six animals were sacrificed from each group at 1,2,4,7,10,14 and 28th day of post irradiation. Blood was collected by heart puncture and complete analysis was done with the help of Sysmex KX-21.

Statistical Analysis

The data were subjected to the Student's 't' test for comparison between control and experimental group for the assessment of significance of difference at the level of P<0.05, P<0.01 and P<0.001.

Results

- (a) **Haemoglobin Content (Hb):** In the control animals, haemoglobin content declined significantly after irradiation in comparison to normal. This decline continued up to 14th post irradiation day. Experimental animals (*Centella asiatica* pretreated animals) also had decrease in their haemoglobin content but this decrease was always lesser in comparison to their respective controls at each autopsy interval. They had early recovery also in hemoglobin. These animals could not attain the normal value up till 28th day post irradiation.
- (b) **Haematocrit Value (Hct):** Haematocrit values declined in both the experimental and control animals. *Centella asiatica* pretreated animals always had higher hematocrit value at each autopsy interval in comparison to their respective control.
- (c) **Red Blood Corpuscles (RBCs):** In irradiated animals red blood cell count declined on 1 day, after treatment then it started to increase up till 14th day post irradiation but failed to attain normal value. The same trend (decline) was observed in *Centella asiatica* pretreated animals, but lesser decrease was observed in comparison to animals irradiated without *Centella asiatica*.
- (d) **White Blood Corpuscles (WBC):** WBC counts also decreased in irradiated animals. On day 10th WBCs completely disappeared. After that on 14th day they started to reappear. The same trend was observed in *Centella asiatica* pretreated animals but a little bit less decrease in comparison to control animals was there. WBC's also failed to attain normal counts within 28 days.

Table-1 Variation in peripheral blood of Swiss albino mice with or without *Centella asiatica* (CA) pretreatment and exposed to 8 Gy of gamma radiation

Haematological parameters	Groups	Post irradiation intervals (in days)						
		1	2	4	7	10	14	28
Hb (%) (g/dl)	8 Gy	5.90±0.01 P<0.001*	6.00± 0.22 P<0.001*	6.40± 0.22 P<0.001*	6.90±0.12 P<0.001*	8.60±0.12 P<0.001*	9.10±0.11 P<0.001*	NS
	8 Gy + CA	6.60±0.01 P<0.001*	7.00±0.01 P<0.001**	7.70±0.03 P<0.001**	9.00±0.05 P<0.001**	10.00±0.07 P<0.001**	11.00±0.08 P<0.001**	13.50±0.09
RBC (x 10 ⁶ mm ³)	8 Gy	3.00±2.5 P<0.001*	3.40±0.02 P<0.001*	3.60±0.02 P<0.001*	5.00±0.15 P<0.001*	5.90±0.19 P<0.001*	6.10±0.09 P<0.001*	NS
	8 Gy + CA	3.20±0.02	3.50±0.4	3.70±0.5	6.00±0.09 P<0.001**	7.90±0.09 P<0.001*	8.50±1.15	10.05±0.30
Hct (%)	8 Gy	17.00±1.0 P<0.001*	17.50±1.12 P<0.001*	19.00±0.99 P<0.001*	20.00±1.5 P<0.05*	25.00±0.98 P<0.001*	28.00±0.09 P<0.001*	NS
	8 Gy + CA	20.00±1.11	20.90±0.12	22.00±1.12	24.00±1.5 P<0.05*	27.00±0.09	30.00±0.09 P<0.001**	39.00±0.08
MCV (μ ³)	8 Gy	62.50±1.29 P<0.0001*	63.00±1.89 P<0.001*	54.00±0.09 P<0.001*	52.50±0.01 P<0.001*	53.80±0.08	50.0±0.09	NS
	8 Gy + CA	47.20±1.12 P<0.001**	47.50±1.05 P<0.001**	52.70±1.00	50.00±1.02 P<0.05**	50.00±0.09 P<0.001**	48.00±0.07 P<0.001**	48.00±0.05
MCH (Pg)	8 Gy	20.60±1.02 P<0.01*	21.00±0.9 P<0.001*	18.00±0.98	17.50±0.98	17.90±0.07	15.20±0.07 <0.001*	NS
	8 Gy + CA	16.20±1.05 P<0.05**	16.50±1.09 P<0.01**	17.70±0.08	16.60±0.09	16.50±0.05 P<0.001**	16.00±0.09 P<0.001**	16.00±1.07
MCHC	8 Gy	33.00±0.02	33.50±0.22	33.50±0.11	33.00±0.12	33.00±0.02	33.30±0.40	NS

Haematological parameters	Groups	Post irradiation intervals (in days)						
		1	2	4	7	10	14	28
(g/dl)	8 Gy + CA	34.70±0.09	35.00±0.08	33.60±0.77	33.00±0.55	33.00±0.04	35.80±0.44	33.87±0.44
WBC (10 ⁶ mm ³)	8 Gy	0.60±0.5 P<0.001*	0.61±0.59 P<0.001*	0.70±0.02 P<0.001*	0.60±0.04 P<0.001*	0	0.80±0.05 P<0.001*	NS
	8 Gy + CA	0.90±0.05	0.99±0.09	1.00±0.08 P<0.01**	0.70±0.12	0	1.20±0.02 P<0.001**	2.10±0.04
Platelets (lakh/cu mm)	8 Gy	7.00±0.91	7.50±0.81	6.50±0.9 P<0.05*	3.50±0.87 P<0.01*	1.50±0.09 P<0.001*	0.70±0.09 P<0.001*	NS
	8 Gy + CA	5.00±0.01	5.60±0.06 P<0.05**	4.85±0.87	2.40±0.87	1.0±0.61	0.50±0.65	3.50±1.0

Values in healthy untreated mouse (Sham irradiated)

RBC – Red Blood Corpuscles 9.50±0.09 (x 10⁶/mm³)

Hb – Hemoglobin Content 14.54 ± 0.10 (g/dl)

HCt – Hematocrit 44.05 ± 1.02 (%)

MCH (Mean Corpuscular Hemoglobin) 16.04±0.20 (pg)

MCV (Mean Corpuscular Volume) 48.0±0.60 (μ³)

MCHC (Mean Corpuscular Hemoglobin Concentration) 33.57±0.45 (g/dl)

Platelets 4.5±0.01 (lakhs/μmm)

WBC 6.42±0.08x10⁶/mm³

P-value Control (8 Gy) vs Normal* Control (8 Gy) vs *Centella asiatica* pretreated**

NS-Not survived

Figure-1 Variation in peripheral blood of Swiss albino mice with or without *Centella asiatica* (CA) pretreatment and exposed to 8 Gy of gamma radiation

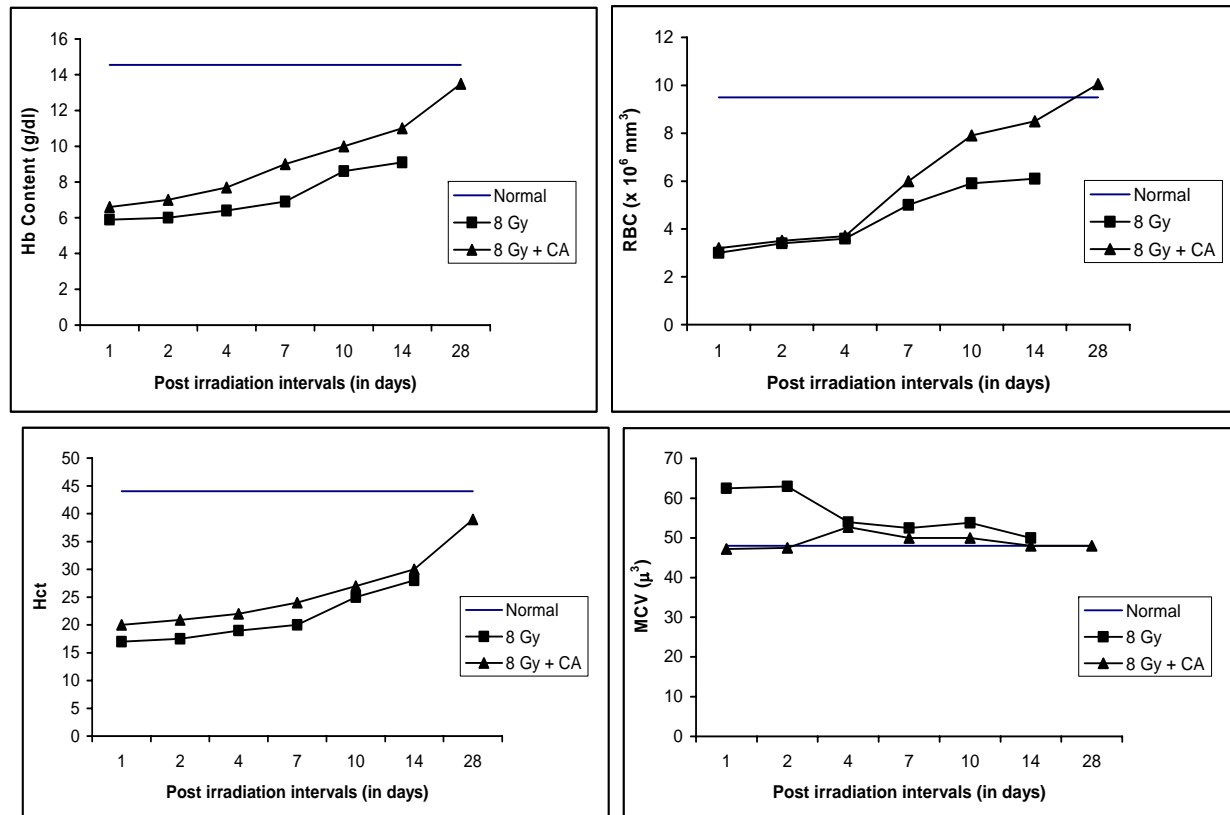
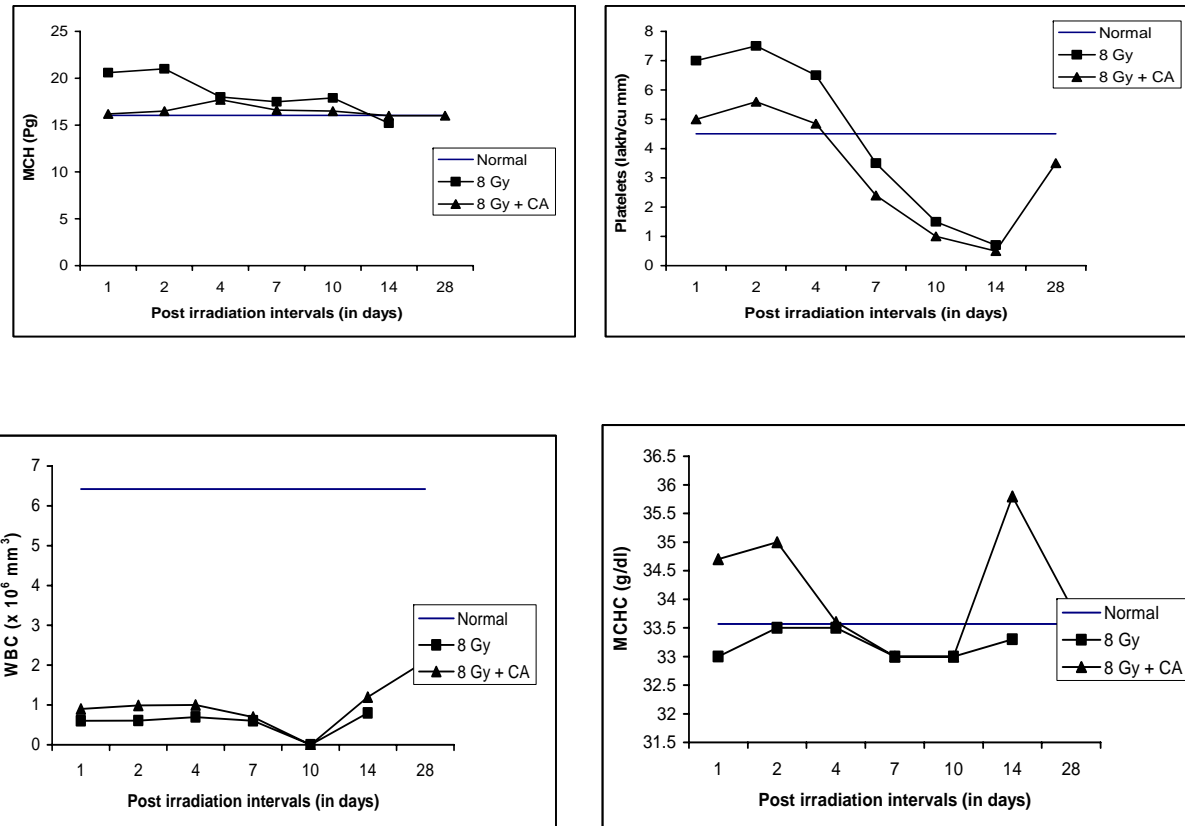


Figure-2 Variation in peripheral blood of swiss albino mice with or without *Centella asiatica* (CA) pretreatment and exposed to 8 Gy of gamma radiation



- (e) **Mean Corpuscular Volume (MCV):** Mean corpuscular volume increased till 2nd post irradiation day which was maximum. From day 4 to 14th post irradiation day it decreased. Reverse trend was observed in the experimental animals. MCV decreased up to 2nd post irradiation day in comparison to both normal and control animals. On day 4th increase in MCV was recorded. After that, from 7th day it decreased and attained normal value on 28th day.
- (f) **Mean Corpuscular Haemoglobin (MCH) :** Mean corpuscular haemoglobin increased up to 2nd post irradiation day, then from 4th day it decreased. Minute increase in MCH value in comparison to both control and normal animals was observed in the experimental mice. These animals attained normal MCH value on day 14 which, continued till 28th day.
- (g) **Mean Corpuscular Haemoglobin Concentration (MCHC):** MCHC was not significantly affected by irradiation in both the control and experimental animals. Very small difference was observed in both the groups.
- (h) **Platelets:** After irradiation platelet counts increased. Maximum increase was observed on day 2nd. From 4th day it started to decrease which continued till 14th day. In the experimental group similar trend was observed but increase was lesser than the control.

Discussion

Hematopoietic tissues are the most radiosensitive components of the body. After whole body exposure, manifestations of radiation injury to mammalian tissues are well reflected in peripheral blood. Ionizing radiation causes decline in the blood cell counts. The quantitative effects of whole body irradiation are more significant than qualitative changes in circulating cells. Exposure to gamma radiation leads to destruction of the blood cells, which are in circulation. Hemorrhage or leakage of the capillary walls and loss of hemopoiesis might have added further.

It was observed in the present study that haemoglobin content and haematocrit values decreased after radiation exposure which seems to be due to the loss of circulating RBC and inhibition of entry of new RBCs as a result of decreased erythropoiesis. This decrease continued further.

Direct damage to the cell membrane leads to destruction of the RBC. Haemoglobin concentration followed a similar pattern to that of RBC. The leakage of RBC into lymphatic tissue and other tissue spaces due to changes in capillary permeability might have added to further loss.

Radiation induced depletion of hematopoietic stem cells is an important factor contributing to the decline in RBC population. After initial decrease hemoglobin content started to increase from 2nd day and this increase continued up to the last interval studied that is 28th day, in both the control and experimental groups. This increase shows beginning of recovery and its continuation in both the groups. In experimental group it touched near normal value. The same trend was observed in the case of RBC counts.

Haematocrit value also decreased significantly on the 1st day after exposure to 8 Gy of Co⁶⁰ gamma rays and it continued to increase up till 28th day in both the control and experimental groups. It seems to be due to the change in the number of RBC at the corresponding intervals, as we know that the packed cell volume (Hct) is mainly dependent on RBC counts. Change in plasma volume may also be a significant factor in early decline in total RBC counts.

It was observed that RBC derived indices like MCH & MCV increased after irradiation. MCV (mean corpuscular volume) increased after irradiation in the control group, which decreased continuously up till 14th day. It is due to radiation induced inflammation of the cells. In the experimental group it remained around the

normal value throughout the experiment, thus showing the protective action of the plant extract. Malhotra *et al*⁶ observed higher value of MCV after irradiation and attributed it to swelling of RBC. Irradiation damages the cell membrane of RBC and changes its permeability, which might have caused swelling. The plant extract pretreatment might have protected the membrane as it is known to prevent radiation induced lipid peroxidation³. This protection to the membrane seems to be the reason of the maintenance of the near normal values of MCV in the experimental animals.

Mean corpuscular haemoglobin (MCH) increased one day after irradiation in the control group but it decreased again on 4th day and continued to decrease upto 14th day, reaching to the near normal value which seems to be due to altered membrane permeability and survival of haemoglobin rich cells. In the experimental group near normal value was retained throughout the duration of experiment. It shows that the direct damage to mean corpuscular haemoglobin is significantly protected by the plant extract pretreatment.

There is no significant change in the mean corpuscular haemoglobin concentration (MCHC) after irradiation in control and experimental groups.

White blood corpuscles (WBC) leucocytes also decrease in number after radiation exposure. In the present study drastic changes in the number of WBC was observed in control as well as experimental animals, showing very high sensitivity of these cells. Damage to the membrane of WBC might have led the WBC to die after radiation exposure since the dose of radiation used in the present experiment is quite high. It would have killed maximum possible number of WBC in the blood. Thus keeping their number to minimum at all the intervals studied. In the plant extract treated group significant increase in the WBC count was observed on 14 and 28th day, which was not visible in the control group. Hence it can be concluded that WBC are also protected by the plant extract pretreatment.

It is well known that radiation exposure reduces the number and functional activity of circulating lymphocytes and changes the distribution and ratio of their subpopulations. RBCs are the cells without nucleus and their death may be attributed to membrane damage. WBCs are well differentiated nucleated cells and DNA damage might have lesser contribution in their death also. Jayirtha *et al.*⁵ (2004) observed that CA treatment significantly increases the phagocytic index and WBC counts.

Blood platelets increased one day after radiation exposure to a great extent and on 7th day their number was below normal and then it decreased again. In the experimental group only a slight increase was observed in the blood platelet count one day after irradiation, but after 4th day their number declined significantly and this decline could not be recovered fully even up to 28th day. It shows that blood platelets are not immediately killed by direct radiation insult. The initial increase in their number may be due to the early maturation/differentiation of the developing platelets, which may be considered as body's response to prevent radiation induced damage. The fall in their number on 7th day and after that might be due to the blockage in the division and differentiation of their stem cells at early intervals. Cells lysis is also induced by irradiation of blood platelets. As soon as mitotic activity of their stem cells is restored, their number reaches to near normal. It happens earlier in the experimental animals. It has been observed that pretreatment of Acetone fraction of *Centella asiatica* at the dose rate of 25 mg/kg body weight protects radiation induced damage to the body, thus increasing survival of animals significantly. According to Srivastava *et al.*⁷ (1997) major constituents of *Centella asiatica* are saponins, triterpenic acids, polyacetylenes, sterols, lipids, alkaloids, hydrocotyline, amino acids, sugars and some flavonoids. Chen *et al.*⁸ found that tetraandrine (separated from *Centella asiatica*) acted against radiation induced acute dermatitis in rats. It might have worked in this case also. *Centella asiatica* extract increases the glutathione level, which is a natural radioprotector secreted by the cells themselves.⁹ Increased glutathione level would have definitely acted to cope up with the harmful effects of radiation. Increased glutathione level also indicate that natural defense mechanisms are activated by *Centella*

asiatica. *Centella asiatica* is known to increase serotonin level and stimulate growth of reticulo-endothelial system, which are also part of natural defense mechanisms.

It has sedative effect and lowers metabolic activities for some time. This sedative effect might also be helpful in exerting its protective effect. *Centella asiatica* contains a group of saponins called asiaticosides that possess strong antioxidant properties. Besides this *Centella asiatica* also contains considerable amount of Ascorbic acid in it, which is also a known antioxidant (Shukla *et al*)¹⁰ and radioprotector. *Centella asiatica* is reported to activate certain enzymatic activities, specifically of xenobiotic enzymes. Asiaticoside stimulates the synthesis of lipids and proteins necessary for healthy skin. *Centella asiatica* extract effectively counteracts alterations in mitochondrial enzymes and mitochondrial defense system in Adriamycin treated rats¹¹.

After a clinical trail with *Centella asiatica*, Apparao¹² found an increase in the mean level of RBC count, blood sugar, serum Cholesterol, vital capacity and total proteins. The increase in haemoglobin percentage was quite high. It also increased mean blood urea level and decreased serum acid phosphatase activity. Recent studies have shown that it has positive effect on the circulatory system, it seems to improve the flow of blood throughout the body by strengthening the veins and capillaries. It has an energizing effect on the cells of the brain, relieves high blood pressure, mental fatigue, senility and helps the body defend itself against various toxins. It works as a blood purifier and promotes blood circulation in the lower limbs and reduces the pain and swelling due to phlebitis.

According to Ponnusamy *et al*¹³ bioflavonoides present in CA are claimed to exert antimutagenic, neurotrophic, and xenobiotics ameliorating and membrane molecular stabilizing effects. CA is found to protect lead acetate induced neurotoxicity. Krishnamurthy *et al*.¹⁴ observed that Asiatic acid, which is a triterpene is also neuroprotective and reduces mitochondrial injury also.

Asiatic acid and asiaticoside inhibits LPS induced NO and PGE(2) production. Asiatic acid reduced production of IL-6, IL-1 beta and TNF alpha in LPS stimulated macrophage cells. Asiatic acid inhibited the NF kappa B activation induced by LPS and this was associated with the abrogation of I-kappa B alpha degradation and with subsequent decreases in nuclear p 65 and p 50 protein levels.¹⁵ *Centella asiatica* extract significantly prevented Adriamycin induced alterations and restored the enzyme activities of tissue antioxidant defense system to near normal levels^{11,20}

Oral administration of aqueous extract of *Centella asiatica* at the dose rate of 100 mg/kg body weight increased the survival time of mouse to a significant extent after 8 Gy of Cobalt-60 gamma radiation.¹⁶ It also protected the mouse against reduction in organ weight, changes in histopathology¹⁷ and alteration in biological parameters.^{16,18}

Oral treatment with 50 mg/kg/day of crude methanol extract of *Centella asiatica* for 14 days significantly increased the antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase¹⁹.

Protection of RBCs and WBCs at early intervals indicates that *Centella asiatica* extract must have protected against radiation induced membrane damage and membrane bound enzymes. Early recovery in *Centella asiatica* pretreated animals shows that precursors of blood cells are also well protected.

From the above findings it can be concluded that acetone extract of *Centella asiatica* has protective activity against deleterious changes produced by ionizing radiations and it has possibility of being used as radioprotector.

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