PROTECTIVE EFFECT OF TINOSPOPRA CORDIFOLIA EXTRACT AGAINST RADIATION INDUCED ALTERATIONS IN PERIPHERAL BLOOD OF SWISS ALBINO MICE

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ABSTRACT
The present investigation has been carried out to evaluate the possible radio protective potential of Tinospora cordifolia root extract (TE) against 8 Gy gamma radiations induced haematological alterations in the peripheral blood of Swiss albino mice. For this purpose, healthy Swiss albino male mice were selected from an inbred colony and divided into four groups. the root extract of Tinospora cordifolia causes an increase in levels of glutathione and vitamin C. glutathione is an important inhibitor of free redical mediated lipid peroxidation and vitamin C is also an excellent hydrophilic antioxidant. Thus, our findings show that pretreatment of Tinospora cordifolia extract before irradiation imparts protective effect on peripheral blood. Tinospora cordifolia alone or in combination offered protection against radiation induced oxidative stress. On the contrary, oral administration of TE before irradiation reduced the radiation-induced variations in all such parameters and the recovery and regeneration was faster as compare to irradiated control group. This investigation indicates that Tinospora cordifolia has the potential to alleviate the radiation mediated adverse effects in peripheral blood and it could be exploited as a protector against planned and unplanned radiation exposure.

Keywords: Gamma Radiation, Radioprotection, Tinospora Cordifolia, Peripheral blood Swiss albino mice.

INTRODUCTION
Tinospora cordifolia a native of India, which is frequently used in various preparations since a long time, is tried to test its radioprotective potential. Infact, Tinospora cordifolia (Miers) is referred as “Amrita” in the ayurved. It is used as general tonic also. Because it is known to treat all the three major doshas referred by Ayurveda “Tridosh shamak”. Tinospora cordifolia a common climber in India is popularly known as “Giloe”. A vary special feature on its structure exists. Its roots are aerial, contain chlorophyll and are photosynthetic. They are green in colour and look like stem. Stem does not exist in this plant. Petiole (leaf stem) and flowering stem sprouts directly from the root and bears leaves and inflorescence. Many texts refer to stem extract which is actually root extract. This root has many typical anatomical features which are characteristic of this plant. This climber is usually grown in the vicinity of “Neem” trees, climbs over it is called as “Neem Giloe”. Another speciality of this is plant is that it is used in serveral herbal combinations. Natural compounds are becoming more popular in radiation research due to their low toxicity, higher efficacy and cost-effectiveness. Plants and herbs contain a plethora of bioactive compounds having antioxidants, anti-inflammatory and immunostimulant
properties which can act either in isolation or in combination to protect against the harmful effects of ionizing radiation.

Species of genus *Tinospora cordifolia* Mires are among the more widely employed medicinal plants throughout a large part of Asia and Africa. In Chinese traditional medicine jinguolan, the very bitter tuberous root of *Tinospora sagittata* has long been known and is commonly used for treating coughs and other throat conditions and used in the folk medicine for various purposes (Bisset and Nwaiwu 1983). The harmful effects of ionizing radiation was discovered ever since the discovery of X-rays by Becquerel in 1896 but the extent of damage was not very clear (Radvanyi & Villain, 2017). The damaging effects of ionizing radiation were more pronounced after the atomic bomb attack in Hiroshima and Nagasaki, Japan in 1945. Since then, the incidence of nuclear terrorism has increased a lot. All these incidences created a global awareness and need to develop a suitable radioprotector. First attempt in the development of radioprotector was made by Patt and his co-workers. They found that the pre-treatment with naturally occurring amino acid cysteine increased the percentage of survival in mice and rats lethally irradiated with X-rays (Patt, Tyree, Straube, & Smith, 1949). However, it was not very successful in clinical trial for human application. Thereafter, several other compounds have been screened and evaluated for their efficacy in rendering radioprotection. However, none of them met the criteria for an ideal radioprotector as of yet.

Ionizing radiations generate free radicals leading to lipid peroxidation, protein oxidation, base modifications, DNA strand breaks and genomic instability ultimately resulting in cell death. In the whole-body irradiated animal, the hemopoietic system is the most susceptible system where cell loss occurs at (8 Gy) doses of exposure. This causes perturbations in the immune system since loss of bone marrow stem cells affects hematopoiesis. At higher doses this effect is more pronounced leading to a total loss of immunocompetent cells and causing immunosuppression that contributes to the emergence of opportunistic infections as observed during the hemopoietic syndrome. The effect of radiation on various cells of the immune system depends on the cell type and the dose of radiation. In this context, *Tinospora cordifolia* (Family: Menispermaceae) a well known plant of Indian medicinal system, is gaining more attention for electing a wide spectrum of pharmacological activities. (Timothy1991) It is known for its general tonic, anti-cancer, anti-leproric, antihyperglycemic, anti-allergic and anti-diabetic properties. It improves the phagocytic and bactericidal capacity of polymorphs, protects against gastric mucosal damage and scavenges free radicals. (Maisin, J. R. (1998) Since this plant has also been reported to possess anti-fibrotic, anti-oxidant, anti-inflammatory, immune modulatory, radio protective and activator of phagocytic and killing activity of macrophages, hence the following study was undertaken to determine whether it can modulate the radiation induced alteration in terms of various hematological and anti-oxidative parameters in experimental animal model.

**MATERIALS AND METHODS**

Animals. Swiss albino mice (6–8 weeks old) weighing 23 ± 2 g were used for the present study. The animals were housed under standard light and dark cycle. Mice were fed on pellet diet (Hindustan Lever, India) and water ad libitum. Irradiation. In the present study *Tinospora condifolla* root extract was used to test its radioprotective efficacy against Co$^{60}$ gamma radiation. A lethal dose (8Gy) of Co$^{60}$ gamma radiation was selected for the purpose. The Cobalt teletherapy unit (ATC-C9) in the cancer treatment centre, SMS Medical College and Hospital Jaipur was used for irradiation. Unanaesthetized animals were restrained in well ventilated perspex and exposed to 8 Gy of 60Co whole body gamma radiation.

**Plant extract.** In the present study *Tinospora condifolla* root extract was used to test its radioprotective efficacy against Co$^{60}$ gamma radiation. Aqueous extract of *Tinospora cordifolia* (dried powder) provided by AMSAR Private Ltd, Indore as a gift sample.

**Experimental design.** Swiss albino mice selected from the inbred colony maintained in the laboratory were divided in to four groups :-
Group I – Animals of this group were sham irradiated. This group served as normal group.
Group II – animal of this group received *Tinospora* extract one hour before irradiation at the dose rate of 5mg/kg body weight orally. Animals were exposed to 8 Gy Co\(^{60}\)gamma radiation. This group served as experimental group.
Group III – Animal of this group were irradiated with 8 Gy Co\(^{60}\)gamma rays and given equal amount of double distilled water as given with the TE and served as control group.
Group IV – Animals of this group received extract of *Tinospora cordifolia* at the dose rate of 5 mg/kg body weight orally.

**Autopsy Intervals**
The animals of all the group were sacrificed by cervical dislocation at ¼, 1,3,5,7,10,14, and 28 days post treatment/irradiation. At least 6 animals of each group were sacrificed at each interval. Testes were removed, weighed and fixed in Bouin’s fluid for histopathological studies.

**Observation**

**Body-weight**
The general condition of the mice in all above group was observed daily and recorded through the measurement of the body weight.

**Survival assay** – Animals of both control and experimental group exposed wholebody to gamma radiation (8Gy) were observed daily for 30 days. The survival percentage of mice up to 30 days exposure against reaiation dose was used to construct survival curve or dose response.

**Weight** of some important vital organs i.e. testis, liver, spleen and thymus was recorded.

**Study of peripheral blood**
Hematological parameters are major indicators of the physiological state of the body. Extensive blood analysis was done with the help of blood analyzer. Blood was collected by heart puncture.

a. RBC (Red Blood Corpuscle)
b. Hb (Haemoglobin)
c. Hct (Haematocrit)
d. MCH (mean corpuscular hemoglobin)
e. MCV (Mean corpuscular volume)
f. (MCHC) (Mean corpuscular hemoglobin concentration)
g. WBC (Total leucocyte counts)
h. Lymphocytes
i. Neutrophils
j. Monocytes
k. Eosionophils
l. Basophils

**RESULT**

**Studies on peripheral blood**

(i) **Total Erythrocyte Count (RBC)**

**Control** – RBC counts decreased after irradiation. RBC counts decreased maximum (3.30 ± 0.06×10\(^6\)/mm\(^3\)) after 3 days following 8 Gy gamma irradiation. Number of RBCs elevated at day 7, but declined subsequently at day 10 without returning to the normal.

**Experimental** – In the experimental group (TE + 8Gy) maximum decrease in total RBC counts was observed at day 3. The RBC counts were significantly higher than their corresponding controls and reached to near normal value within day 5 to day 10 (Table -1).
(ii) Haemoglobin (Hb)
Control – Hb concentration showed a significant decrease, first at 6 hours post irradiation with a gradual recovery till day 10.
Experimental – A gradual recovery in haemoglobin content was evident from 6 hrs. onwards. However, a significant increase was observed at almost all the autopsy intervals as compared to their respective controls (Table -1).

(iii) Haematocrit Value (Hct %)
Control – The Hct percentage was found to decrease in these animals after irradiation which could not reach to the normal within 10 days time.
Experimental – Haematocrit percentage was decreased but to a lesser in this group. A significant recovery was observed but normal value could not be attained even till 10th post – irradiation day normal (Table – 1).

(iv) Mean corpuscular haemoglobin (MCH)
Control – A significant rise over normal in the values of MCH was observed in these animals exposed to 8 Gy gamma radiation, which continued up to day 3 finally to go down on day 7 and increased again at day 10.
Experimental – Experimental animals showed a very little increase in the values of MCH in animals pretreated with TE and exposed to gamma radiation (8Gy). But these values remained significantly lower as compared to respective controls at each autopsy interval (Table -1).

(v) Mean corpuscular volume (MCV)
Control – A significantly higher value of MCV was recorded at day 1 after autopsy but afterwards, a gradual decline was evident and the values were significantly lesser than normal at day 7 and day 10.
Experimental – MCV values were significantly lower as compared to respective controls at each autopsy interval. However, a decrease was observed with first decline at 6 hrs. and second at day 7. Normal range could not be obtained till day 10 post-irradiation (Table -1).

(vi) Mean corpuscular haemoglobin concentration (MCHC)
Control – In this group MCHC values found to increase significantly following 8Gy gamma irradiation. The peak value of MCHC was observed at day 10 post irradiation.
Experimental – Maximum increase in MCHC value was observed at day 3 and than after, a gradual decrease was noticed on day 10 post-irradiation. All the values were found to be significantly below to their respective controls at each autopsy interval (Table-1).

(vii) Total leucocyte count (WBC)
Control – Decrease in WBC count was noted as early as at 6 hrs. post-irradiation and which could not be normal till day 10 in this group after irradiation.
Experimental – WBC counts were observed significantly higher than the control animals. Although still they are not equal to the normal ones. A significant increase in WBC counts was evident at 1/4th day onwards up to day 10 post irradiation reaching towards normal (Table -2).

Differential Leucocyte Count (DLC)
(i) Lymphocytes
Control – A significant drop in lymphocyte percentage was noted in this group from first autopsy interval (6hrs.) and the minimum value was recorded at day 7 post irradiation. After this, lymphocyte count increased on day 7 but depleted again at day 10.
Experimental– A significant increase over control was observed in lymphocyte percentage on 4th day. However, the decrease in lymphocyte was a little lesser than the control. At day 10, a significant increase over control was noticed(Table -2).

(ii) Neutrophils
Control – Neutrophil percentage elevated at first autopsy interval (6hrs.) and the maximum increase in these cells was recorded at day 5 post-irradiation and continued upto day (Table-4).
Experimental– In this group an increased percentage of neutrophils was observed but this increase in less although not significant in comparison to the controls. A significant decrease as compared to the control was evident at day 10.

(iii) Monocytes
Control– The number of monocytes increased at 6 hrs. after irradiation but at later intervals a decrease in monocyte percentage was recorded.
**Exprimental**–A significant decline in monocyte percentage was observed at 6 hrs interval, very significant variation was noticed during the entire period of study between control and experimental animals (Table-2).

**Eosinophils**

**Control** – A biphasic increase was observed in the eosinophil percentage. It increased at 6 hrs, 3 day and 10 days after irradiation while its lower values are observed at day 5 and day 7.

**Experimental**- The number of eosinophils decreased initially at ¼ day and day 1. It increased at 3 to decline further at day 5 and day 7. It increased again at day 10. Although the values are always lower in comparison to the controls (Table- 2).

**Basophils** –

**Control**–Basophil percentage did not show any significant variations as compared to the normal at day ¼. An increase was recorded at day 1 and 3 post irradiation but at day 10 very high increase in basophil percentage was recorded.

**Experimental**–Basophils percentage showed variation from the controls. After day 5 values of such cells remained below the normal (Table-2)

**DISCUSSION**

Irradiation to 8 Gy gamma radiation which is a lethal dose, leads to the destruction of blood cells, which are in circulation. It may be added further by hemorrhage or leakage to the capillary walls and loss of hemopoiesis (Casarett 1968).

It was observed that haemoglobin content, number of circulating RBCs and hematocrit values decreased after radiation exposure. It is due to the loss of circulating cells and inhibiton of new entry of the RBCs. This decrease continues further. According to Stohalaman et al. (1957). Decrease after irradiation is due to damage to the RBCs and loss by hemorrhage. According to Fred and Smith (1968) radiation induced depletion of haematopietic stem cells may be an important factor contributing to the decline in RBC population. Direct damage to the cells and their membrane leads to destruction of the RBCs. The hemoglobin content also decreases following radiation exposure (Shaheen and Hassan, 1991). Haemoglobin concentration followed a similar pattern to that of RBCs after irradiation. It is understood that decreased hemoglobin content is due to decrease in the number of RBCs. Haematocrit values also decrease after exposure to 8 Gy of gamma radiation and it may be due to destruction of mature cells and internal bleeding (Soberman et al. 1951, Malhotra and Srivastava, 1975). It was also observed that RBC derived indices like MCH, MCV and MCHC values increased after radiation exposure. Higher values of MCV were attributed to swelling of RBCs (Malhotra et al. 1990). Decreased rate of effective erythropoiesis and early death of RBCs also lead to anemia (Block 1976). Increase in MCHC may be due to normal maintenance of regular haemoglobin synthesis.

WBCs also decrease in number after radiation exposure (Jacobson et al. 1947). Differential count of WBCs indicates that all the types of WBCs except neutrophils decrease in number after irradiation. It may be due to direct decrease of these cells in the peripheral blood (Patt 1967). Lymphocytes were the most radiosentive amongst these.

The neutrophils increased 6 hrs after irradiation and this increase was continued. This increase may be due to abortive rise phenomenon (Bloom and Jacobson, 1948). Early maturation of granulocyte precursors in bone marrow and their release in circulation may be another factor (Wuensche, 1938).

Neutrophils are phagocytic cells. When they are activated during phagocysis, they generate O$_2$- and H$_2$O$_2$through NADPH oxidase. Neutrophil accumulates in the inflamed tissue and oxidative damage due to generation of ROS occurs to the tissue (Bondyopadhyay et al., 1999). It is one of the reason of the increased neutrophils after irradiation while lesser number of neutrophil in TE pretreated animals can be attributed to lesser inflammation and damage in those animals.

In the present study, it was observed that TE pretreated and irradiated animals showed significant increase in the total number of erythrocyte, leucocytes, hemoglobin content and haematocrit value as compared to their respective controls at all the post irradiation intervals.

In the TE pretreatment before irradiation showed a significant protection to haematological constituents against gamma radiation. The recovery in blood cells appeared due to recovery of haematopoietic organs which supply new cells for peripheral circulation.
Sutherland and Pihl (1968) noticed that the deflected RBC ghost are more prone to radiation than intact red blood cells because such cells have more proteins to protect –SH. The decrease in interior glutathione of irradiated cells may be related to diminished ability to restore S-S group to their original states. Stanely and Menon (1999) suggested that the root extract of *Tinospora cordifolia* causes an increase in levels of glutathione and vitamin C. glutathione is an important inhibitor of free radical mediated lipid peroxidation and vitamin C is also an excellent hydrophilic antioxidant. Thus, our findings show that pretreatment of *Tinospora cordifolia* extract before irradiation imparts protective effect on peripheral blood. *Tinospora cordifolia* alone or in combination offered protection against radiation induced oxidative stress. Oxidative stress refers to the cytotoxic consequence of free radicals (O₂, OH and H₂O₂), which are generated as byproducts of normal and aberrant metabolic process (Coyle and Puttfarcken, 1993). Exposure to oxidative results in the progressive degeneration of membrane structure and loss of activity the protective effect observed in the present work may be explained due to the scavenging of oxidising free radicals. *Tinospora cordifolia* is reported to process antioxidant property (Stanely *et al.* 2001).

On the basis of above discussion it can be concluded that *Tinospora cordifolia* protects swiss albino mouse to a significant extent. Not only this, it significantly protects the testes also. There are several mechanisms by which chemicals and other radioprotector protect an organism, organ, tissue or cells, usually are:

1. antioxidants
2. free radical scavengers and
3. donor of hydrogen atom.

In this they may be creators of hypoxia and reducers of blood supply, ultimately leading to lowered oxygen tension. Sometimes they interact with cellular components and prevent lipid peroxidation. Some of them bind with DNA and prevent DNA strand breaks.

In all these ways cellular molecules are protected and specific enzymatic machinery remains active finally to repair the damage caused to the cellular micro molecules. After radiation lesions defense mechanisms of the body also become active and secrete protective chemicals to get the body out of radiation shock. The major difference between different classes of chemicals and plant extracts, is that plant extracts are mixture of organic and inorganic compounds. Most of the plants contain flavonoid, phytohormones, vitamins and nutrients. *Tinospora cordifolia* root contains alkaloids, glycosides, sterols, lactones and fatty acids. The major subtanses separated from it are berberine, tinosporin, giloinin, giloin and giloisterol. It is reported to enter in almost all the tissues of the body.
Table 1: Variation in peripheral blood of Swiss albino mice with or without TE pretreatment and exposed to 8 Gy of gamma irradiation.

<table>
<thead>
<tr>
<th>Hematological Parameter</th>
<th>Group</th>
<th>Post irradiation time (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4</td>
</tr>
<tr>
<td>RBCs X10^6 mm^-3</td>
<td>Control</td>
<td>4.20±0.07**</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>7.56±0.19</td>
</tr>
<tr>
<td>Hb (gm/dl)</td>
<td>Control</td>
<td>8.75±0.05**</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.50±0.14</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>Control</td>
<td>21.60±0.10*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.20±1.29</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>Control</td>
<td>21.90±0.42*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.50±0.45</td>
</tr>
<tr>
<td>MCV (µ³)</td>
<td>Control</td>
<td>55.80±1.52*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40.50±0.70</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>Control</td>
<td>40.50±0.36*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>43.50±1.23</td>
</tr>
</tbody>
</table>

Values in untreated healthy mouse:

- RBC = 9.35±0.06x10^6/mm³
- Hb = 14.89±0.90 gm/dl
- Hct = 41.90±0.20%

Additional hematological parameters:

- MCH = Mean Corpuscular Volume
- MCV = Mean Corpuscular Hemoglobin Concentration

Abbreviation:

- RBC = Red Blood Corpuscle
- Hb = Hemoglobin
- Hct = Hematocrit
- MCH = Mean Corpuscular Hemoglobin
- MCHC = Mean Corpuscular Hemoglobin Concentration

Significance level:

- *=P<0.05
- ** =P<0.01
- *** =P<0.001
Table 2 Variation in total leucocyte counts and differential leucocyte counts in peripheral blood of Swiss albino mice with or without TE pretreatment and exposed to 8 Gy of gamma irradiation.

<table>
<thead>
<tr>
<th>Hematological Parameter</th>
<th>Group</th>
<th>Post irradiation time (in days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/4</td>
</tr>
<tr>
<td>WBC (10^6/mm^3)</td>
<td>Control</td>
<td>4.20±0.07**</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>5.90±0.30</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>Control</td>
<td>49.5±1.50*</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>56.5±1.30</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>Control</td>
<td>43.2±0.50***</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>39.5±0.60</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>Control</td>
<td>3.8±0.40**</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>1.2±0.40</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>Control</td>
<td>4.2±0.60</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>2.1±0.20</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>Control</td>
<td>0.5±0.30</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.7±0.31</td>
</tr>
</tbody>
</table>

Values in untreated healthy mouse
WBC = 6.42±0.08 X 10^6/mm^3
Lymphocytes = 68.5±2.50%
Neutrophils = 22.5±2.30%

REFERENCES


