

## The Ameliorative Effect of Echinacea Purpurea Against Gamma Radiation Induced Oxidative Stress and Immune Responses in Male Rats

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**Abstract:** While radiation hazards, due to free radical generation, present an enormous challenge for biological and medical safety, Echinacea purpurea is a potent scavenger of a variety of free radicals. The aim of this study was to investigate the radioprotective effect of Echinacea purpurea against oxidative stress in spleen tissues and alterations in some immune responses. Rats were whole body  $\gamma$ -irradiated with 3Gy/week up to a total dose of 6Gy. E. purpurea (30mg/Kg body weight/day) was given to rats, via gavages, during 14 days before irradiation and 7 days after each radiation dose. Animals were sacrificed on the 7<sup>th</sup> day after the last radiation dose. A significant increase in xanthine oxidase (XO) activity along with a significant decrease in xanthine dehydrogenase (XDH) activity was recorded in the spleen of irradiated rats. In addition, the levels of oxidative biomarkers; thiobarbituric acid reactive substances (TBARS), protein carbonyl and advanced oxidation protein products (AOPP) showed a significant increase. The activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) showed significant increases, while glutathione peroxidase (GSHPx) activity showed a significant decrease and glutathione content showed normal value in the spleen of irradiated rats. Irradiation resulted also in significant decreases in total white blood cells (WBCs) count, lymphocytes, monocytes, neutrophils, eosinophils, basophils and potassium level. On the other hand, a significant increase in the level of sodium and C-reactive protein (CRP) was recorded in the serum of irradiated rats. E. purpurea supplementation has significantly improved oxidative stress in the spleen of irradiated rats. Total and differential white blood cells (WBCs) count showed a significant increase compared to their corresponding values in irradiated rats while serum CRP returned normal. It could be concluded that E. purpurea may afford protection against radiation-induced oxidative stress in spleen tissues and is involved in the modulation of immune responses.

**Key words:**  $\gamma$ -rays, Echinacea purpurea, spleen, oxidative stress, sodium, potassium, C-reactive protein, WBCs.

### INTRODUCTION

Since the discovery of X-ray 100 years ago, radiation has been used increasingly in medicine and industry to help with diagnosis, treatment, and technology. Radiations have tremendous therapeutic benefits for human. However, it is also associated with the risk of serious adverse effects (Borek, 2004). The deleterious effects of ionizing radiation are associated with alteration in the xanthine oxidoreductase (XOR) system through the conversion of xanthine dehydrogenase (XDH) into xanthine oxidase (XO) and an increase of superoxide anion ( $O_2^{\cdot-}$ ) (Srivastava *et al.* 2002). Superoxide anion can be converted spontaneously or enzymatically into hydrogen peroxide ( $H_2O_2$ ) and then into the highly reactive hydroxyl radical ( $\cdot OH$ ) that initiate the lipid peroxidation chain reaction (Candeias *et al.*, 1995). Superoxide anion may also react with nitric oxide (NO) to generate the cytotoxic peroxynitrite anion ( $ONOO^{\cdot-}$ ), which can react with carbon dioxide, leading to protein damage via the formation of nitrotyrosine (Huie and Pasmaja 1993).

Exposure to ionizing radiation leads to the generation of extra reactive oxygen species and free radicals which attack sensitive enzymes, constitutive proteins, DNA and membrane lipids (Blatter and Herrlich, 2004). Evidence for oxidative injury is proved from measurements of biochemical markers of lipid peroxidation and protein oxidation. Lipid peroxidation is believed to be an important cause of destruction and damage to cell membranes and has been shown to be a contributing factor to the development of oxygen radicals-mediated tissue damage. Altered protein molecules can act as traps for chemical energy released by free radicals and initiate further chain reactions, thus enhancing the damage as observed with lipid peroxides. Advanced oxidation protein products (AOPP) are reliable markers of the degree of protein damage in oxidative stress (Simpson *et al.*, 1993, Witko-Sarsat *et al.*, 1999). However, cells are equipped with several natural enzymatic and non-enzymatic antioxidant defenses (Halliwell, 1992). The exposure of human body to ionizing radiation leads to depletion of these endogenous antioxidants and ultimately to the development of systemic disease (Koc *et al.*, 2003).

Many natural and synthetic compounds have been investigated for their efficacy to protect against irradiation damage (Nair, 2004). Moreover, a potential treatment strategy for radiation exposure might be to strengthen the immune system (Gunsilius *et al.*, 2001). Echinacea purpurea, also known as the purple

coneflower is a member of the Asteraceae (daisy) family. It is one of the most popular herbal medicines that contain many active components such as alkamides, caffeic acid esters (cichoric acid), polysaccharides and polyacetylenes. *Echinacea purpurea* has been reported to possess anti-tumor (Block *et al.*, 2002) and anti-inflammatory activities (Raso *et al.*, 2002). Additionally, *E. purpurea* was found to possess antioxidant properties (Rininger *et al.*, 2000, Pellati *et al.*, 2005) making it a very promising medicinal botanical (Dalby-Brown *et al.*, 2005 and Chen *et al.*, 2005). Its popularity today is fuelled by claims that it has immune stimulating properties and can reduce the severity of symptoms and duration of the common cold and flu, particularly if used in the early stages of infection (Barrett, 2003).

The hematopoietic system is known to be one of the most radiosensitive systems, and its damage lead to the development of hematopoietic syndrome. Death from the so-called hematopoietic syndrome results from infection due to the impairment of the immune system (Chen *et al.*, 2006). Various mechanisms such as the prevention of damage through the inhibition of free radical generation or its intensified scavenging, enhancement of DNA and membrane repair, replacement of dead hematopoietic and other cells and the stimulation of immune-cells activities are considered to be important approaches for radio-protection and radio-recovery (Nübel *et al.*, 2006).

The spleen plays an important role in immune function by trapping and processing antigens, homing, transforming and proliferating lymphocytes and activating macrophages (Witztum, 2002). Immune responses include innate responses and adaptive responses. Innate responses use neutrophils, monocytes, basophils, and eosinophils and acute-phase proteins such as C-reactive protein (CRP). Adaptive responses involve B and T lymphocytes. Innate and adaptive responses usually work together (Delves and Roitt, 2000). Therefore, the aim of the present study was to evaluate the radioprotective effects of *Echinacea purpurea* counteracting oxidative damage and alternation of some immune response in spleen.

## MATERIALS AND METHODS

### **Administration of *E. Purpurea*:**

Standardized dried powder extract from *E. purpurea* (Echinacin; Madaus AG, Germany) at a dose of 30 mg/kg body wt/day, was suspended in 1.0 ml of saline and gavaged to each animal for 4 weeks as previously described by (Di Carlo *et al.*, 2003). The dried powder extract from *E. purpurea* includes caffeic acid derivatives (primarily echinocside), flavonoids, essential oils, polyacetylenes, alkylamides and polysaccharides (Kim *et al.*, 2002).

### **Animals:**

Male albino rats with an average weight of 100-120 g were obtained from the Holding Company for Biological Products and Vaccines, Cairo, Egypt. The animals were kept under good ventilation, at a temperature of  $22 \pm 3^{\circ}\text{C}$ , 60% humidity, and suitable illumination conditions (light/dark cycle) and allowed standard pellet diet and fresh water ad libitum.

### **Irradiation:**

Irradiation was performed through the use of a Canadian Gamma Cell-40 ( $^{137}\text{Cs}$ ) at the National Center for Radiation Research and Technology (Cairo, Egypt). The dose rate was 0.6 Gy/minute.

### **Study Design:**

Animals were divided into four groups (n=8) as follows:

- Control: rats didn't receive any treatment.
- *E. purpurea*: Each rat was given an appropriate dose of *E. purpurea* suspension/day during four weeks.
- -Irradiated: Animals were subjected to fractionated dose of whole body  $\gamma$ -rays (3Gy/week) for 2 weeks.
- Irradiated + *E. purpurea*: Animals received *E. purpurea* dosage/day for 2 weeks before irradiation and through the period of radiation exposure.

### **Blood Collection and Tissue Sampling:**

Animals were sacrificed on the 7<sup>th</sup> day after the last gamma radiation dose. Blood samples were collected by heart puncture. Spleen was quickly removed, rinsed thoroughly from blood in isotonic saline, blotted dry, and weight then homogenized in saline solution. The homogenate was centrifuged at 3000 rpm for 15 min and the supernatant was used for biochemical analysis.

### **Biochemical Study:**

XOD and XDH activities were determined according to the procedures of Kaminski and Jewezska (1979). Superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT) activities were determined

according to the methods of Minami and Yoshikawa (1979), Paglia and Valentine (1967), and Sinha (1972), respectively. The content of glutathione was determined according to the method of Beutler (1963). Lipid peroxidation was determined as thiobarbituric acid-reactive substances (TBARS) as described by Yoshioka *et al.*, (1979). Advanced oxidation protein products (AOPP) was measured according to Witko-Sarsat *et al.*, (1999). Protein carbonyl value was determined according to Reznick and Packer (1994). Total and differential WBCs count were determined using Sysmex (KX-21-cell counter), with a Kit manufactured by Diamond, Philadelphia, (USA). Plasma levels of the inflammatory biomarker C-reactive protein (C-RP) titer were measured using a sensitive latex particle- enhanced immune turbidimetric assay (Kamiya Biomedical company) as described by Roberts *et al.*, (2001). Serum sodium and potassium levels were assayed by flame photometry (Marshall, 1995) using JENWAY PFP-7 Flame Photometer (England) All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) USA. The kits used were purchased from Bio-diagnostic.

#### Statistical Analysis:

The SPSS 15.0 statistical software package programmed for Windows was used for statistical calculations. Data were analyzed using one way analysis of variance (ANOVA). Post-hoc Duncan test was used to determine significant differences between means. Values were expressed as mean  $\pm$  SE. (n=6). Differences between means were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSIONS

The results obtained in the present study revealed that supplementation of *E. purpurea* to rats during a period of 4 weeks did not affect the XOR system (table 1), as well as, the oxidant/antioxidant status of the spleen (tables 1 and 2). Furthermore, serum electrolytes (table 3) as well as total and differential WBCs count and serum C-reactive protein (table 4) were within the normal levels.

Table 1: shows that the whole body exposure of rats to gamma radiation induced significant decrease in the activity of xanthine dehydrogenase in parallel to significant increase in xanthine oxidase activities, TBARS, AOPP, and protein carbonyl levels compared to their corresponding values in the control group. Supplementation of rats with *E. purpurea* before and after whole body gamma irradiation has significantly improved the xanthine oxidoreductase system in addition to a significant depression in TBARS, AOPP, and protein carbonyl levels compared to irradiated group was attained upon *E. purpurea* administration combined to irradiation.

Table 2: demonstrate significant increases in the activity of SOD and CAT, while a significant decrease was recorded in the activity of GSHPx, and no significant change in GSH content, compared to their corresponding values in the control group. Supplementation with *E. purpurea* has significantly ameliorated the antioxidant status of irradiated rats.

Table 3: illustrates that  $\gamma$ -irradiation significantly increased serum sodium concentration and significantly decreased potassium concentration as compared to the control level. Supplementation with *E. purpurea* significantly altered their concentrations returning potassium to normal level.

Table 4: showed that total white cell, lymphocytes, neutrophils and monocytes were significantly lower in irradiated rats, compared with their corresponding levels in the control group. *E. purpurea* supplementation has significantly ameliorated these decreases. C- reactive protein increased significantly in irradiated group, compared to its level in the control group. It returned to the normal level in rats supplemented with *E. purpurea*.

**Table 1:** Influence of *Echinacea purpurea* and/or gamma-irradiation on xanthine oxidoreductase system, TBARS, AOPP and protein carbonyl in the spleen of different rat groups.

| Groups  | Control         | <i>E. purpurea</i>       | Irradiated                             | Irradiated+<br><i>E. purpurea</i>       |
|---|-----------------|--------------------------|--|---|
| Xanthine Oxidase mU/mg protein %<br>Change from control       | 1.20 $\pm$ 0.10 | 1.22 $\pm$ 0.13<br>↑1.5  | 2.39 $\pm$ 0.17 <sup>a</sup><br>↑100   | 1.79 $\pm$ 0.15 <sup>abc</sup><br>↑50   |
| Xanthine Dehydrogenase mU/mg<br>protein % Change from control | 2.7 $\pm$ 0.3   | 3.0 $\pm$ 0.15<br>↑10.6  | 1.35 $\pm$ 0.14 <sup>a</sup><br>↓50    | 1.56 $\pm$ 0.14 <sup>ab</sup><br>↓25    |
| TBARS $\mu$ mol/g tissue % Change from<br>control             | 209.1 $\pm$ 7.9 | 205.5 $\pm$ 8.4<br>↓1.7  | 288.8 $\pm$ 9.5 <sup>ab</sup><br>↑38.1 | 245.5 $\pm$ 7.8 <sup>abc</sup><br>↑17.4 |
| AOPP $\mu$ mol/g tissue % Change from<br>control              | 0.52 $\pm$ 0.06 | 0.46 $\pm$ 0.05<br>↓11.5 | 0.78 $\pm$ 0.06 <sup>a</sup><br>↑50    | 0.84 $\pm$ 0.07 <sup>a</sup><br>↑61     |
| Protein carbonyl nmol/mg protein %<br>Change from control     | 0.69 $\pm$ 0.07 | 0.66 $\pm$ 0.04<br>↓4.3  | 1.35 $\pm$ 0.12 <sup>ab</sup><br>↑95.7 | 0.95 $\pm$ 0.1 <sup>abc</sup><br>↑37.7  |

Each value represents the mean  $\pm$  SE (n=8).

<sup>a</sup> significant difference compared to control group.

<sup>b</sup> significant difference compared to *E. purpurea* treated group.

<sup>c</sup> significant difference compared to irradiated group.

**Table 2:** Effect of *E. purpurea* on activity of enzymatic antioxidants SOD, CAT, GSHPx and GSH content in the spleen of different rat groups.

| Groups  | Control          | E. purpurea               | Irradiated                               | Irradiated + <i>E. purpurea</i>       |
|---|------------------|---------------------------|--|---------------------------------------|
| SOD $\mu\text{g/g}$ tissue %Change from control                                       | 286.7 $\pm$ 27.4 | 276.7 $\pm$ 29.1<br>↓3.49 | 341.9 $\pm$ 17.2 <sup>ab</sup><br>↑19.25 | 308.2 $\pm$ 31.1 <sup>c</sup><br>↑7.5 |
| CAT $\mu\text{mol/g}$ tissue %Change from control                                     | 0.73 $\pm$ 0.04  | 0.69 $\pm$ 0.02<br>↓5.5   | 0.94 $\pm$ 0.03 <sup>ab</sup><br>↑28.8   | 0.81 $\pm$ 0.06 <sup>c</sup><br>↑11   |
| GSH-Px $\square\text{g}$ consumed GSH/ $\text{g}$ wet tissue/min %Change from control | 470 $\pm$ 25     | 488 $\pm$ 28<br>↑3.8      | 400 $\pm$ 26 <sup>ab</sup><br>↓14.9      | 447 $\pm$ 32 <sup>c</sup><br>↓4.9     |
| GSH $\text{mg/g}$ tissue %Change from control   | 11.31 $\pm$ 1.1  | 11.99 $\pm$ 0.8<br>↑6.0   | 11.69 $\pm$ 0.9<br>↑3.4                  | 11.52 $\pm$ 1.2<br>↑1.9               |

Each value represents the mean  $\pm$  SE (n=8).

<sup>a</sup> significant difference compared to control group.

<sup>b</sup> significant difference compared to *E. purpurea* treated group.

<sup>c</sup> significant difference compared to irradiated group.

**Table 3:** Effect of *E. Purpurea* and/or gamma-irradiation on Na<sup>+</sup> and K<sup>+</sup> levels in serum of different rat groups.

| Groups                                       | Control         | E. purpurea             | Irradiated                             | Irradiated + <i>E. purpurea</i>         |
|--|-----------------|-------------------------|--|---|
| Na <sup>+</sup> m mol/L %Change from control | 147.5 $\pm$ 4.8 | 143.5 $\pm$ 4.1<br>↓2.7 | 236.1 $\pm$ 4.7 <sup>ab</sup><br>↑60.1 | 202.2 $\pm$ 6.2 <sup>abc</sup><br>↑37.1 |
| K <sup>+</sup> m mol/L %Change from control  | 5.64 $\pm$ 0.32 | 5.02 $\pm$ 0.34<br>↓11  | 4.25 $\pm$ 0.21 <sup>ab</sup><br>↓25.4 | 5.42 $\pm$ 0.41 <sup>c</sup><br>↓3.9    |

Each value represents the mean  $\pm$  SE (n=8).

<sup>a</sup> significant difference compared to control group.

<sup>b</sup> significant difference compared to *E. purpurea* treated group.

<sup>c</sup> significant difference compared to irradiated group.

**Table 4:** Influence of *Echinacea purpurea* and/or gamma-irradiation on total and differential WBC's count  $\times 10^3/\mu\text{l}$  and serum C- reactive protein (mg/dl).

| Groups                            | Control         | E. purpurea              | Irradiated                             | Irradiated + <i>E. purpurea</i>        |
|-----------------------------------|-----------------|--------------------------|--|--|
| WBCs %Change from control         | 7.44 $\pm$ 2.10 | 7.21 $\pm$ 0.41<br>↓3.1  | 3.78 $\pm$ 0.24 <sup>ab</sup><br>↓49.2 | 5.89 $\pm$ 0.5 <sup>abc</sup><br>↓20.8 |
| Lymphocytes % Change from control | 2.69 $\pm$ 0.32 | 2.74 $\pm$ 0.19<br>↑1.86 | 1.77 $\pm$ 0.16 <sup>ab</sup><br>↓34.2 | 2.21 $\pm$ 0.3 <sup>abc</sup><br>↓17.8 |
| Monocytes % Change from control   | 0.40 $\pm$ 0.02 | 0.43 $\pm$ 0.04<br>↑7.5  | 0.20 $\pm$ 0.01 <sup>ab</sup><br>↓50   | 0.28 $\pm$ 0.02 <sup>abc</sup><br>↓30  |
| Neutrophils % Change from control | 3.36 $\pm$ 0.41 | 3.46 $\pm$ 0.27<br>↑2.98 | 2.35 $\pm$ 0.41 <sup>ab</sup><br>↓30.1 | 2.86 $\pm$ 0.3 <sup>abc</sup><br>↓14.9 |
| Eosinophils % Change from control | 0.20 $\pm$ 0.01 | 0.21 $\pm$ 0.02<br>↑5    | 0.13 $\pm$ 0.02 <sup>ab</sup><br>↓35   | 0.15 $\pm$ 0.01 <sup>ab</sup><br>↓25   |
| Basophils % Change from control   | 0.07 $\pm$ 0.03 | 0.069 $\pm$ 0.01<br>↓1.4 | 0.05 $\pm$ 0.01 <sup>ab</sup><br>↓29.6 | 0.056 $\pm$ 0.02 <sup>ab</sup><br>↓20  |
| Serum CRP % Change from control   | 0.36 $\pm$ 0.09 | 0.37 $\pm$ 0.05<br>↑2.8  | 0.66 $\pm$ 0.04 <sup>ab</sup><br>↑83.3 | 0.38 $\pm$ 0.06 <sup>c</sup><br>↑5.6   |

Each value represents the mean  $\pm$  SE (n=8).

<sup>a</sup> significant difference compared to control group.

<sup>b</sup> significant difference compared to *E. purpurea* treated group.

<sup>c</sup> significant difference compared to irradiated group.

### Discussion:

Exposure to ionizing radiation whether occupational or during radiotherapy leads to serious systemic damage to various cellular and subcellular structures. Radiation exposure creates free radicals causing oxidative stress where antioxidant activity declines and lipid peroxidation increases (Nordberg and Arner, 2001).

The results obtained in the present study demonstrated that rats receiving 30mg *E. purpurea* via gavages during 4 weeks showed no significant changes in XOR system and oxidant: antioxidant status in the spleen. Furthermore, no significant changes were recorded in total and differential WBCs count. The content of serum CRP showed normal values. The results are consistent with available data revealing *E. purpurea* as safe and without significant toxicity, or adverse side effects (Block and Mead, 2003).

On the other hand, whole body  $\square$ -irradiation of rats with 3Gy/week up to a total dose of 6Gy induced a significant increase in spleen XO activity along with a significant decrease in XDH activity, compared with control rats. Alteration in XOR system was associated to significant increases in oxidative biomarkers; AOPP, TBARS, and protein carbonyl level. The results are compatible with previous findings that ionizing radiation induces the conversion of XDH into XO (Srivastava *et al.*, 2002). The enhanced specific XO activity induces oxidative stress whereby the excess of free radicals interact with various components of the cell and resulted in elevation of oxidative products.

In the present study, the significant increase of oxidative biomarkers was associated with alternation in the antioxidant status of the spleen. The significant increase in the activity of SOD and CAT might be attributed to increased expression of these enzymes as a self-defense mechanism against oxidative stress (Guo *et al.*, 2003). On the other hand, a significant decrease was recorded in GSHPx activity which could be attributed to the uncontrolled production of ROS and accumulation of  $H_2O_2$  whereby oxidative damage to enzymes can cause a modification of their activity (Kregel and Zhang, 2007). *E. purpurea* (30mg/Kg body weight/day) given to rats via gavages during 14 days before irradiation and 7 days after each radiation dose has significantly ameliorated oxidative stress in spleen tissues. This could be attributed to the presence of echinacoside and caffeic acid in *E. purpurea* which are potent scavengers of free radicals such as hydroxyl radicals produced by irradiation reducing cellular injury and preventing cellular membrane destruction by oxidation (Mishima *et al.*, 2004). Weiss and Landauer (2003) related the protective effect of *E. purpurea* to the presence of polyphenols and a class of specific antioxidants known as caffeoyl derivatives.

Eliminated lipid peroxides in irradiated rats are quite correlated with the disturbance in the concentration of  $Na^+$  and  $K^+$  (Abou Safi *et al.*, 2006). They found that cell injury following irradiation was associated with disturbance in the cell membrane permeability as exhibited by changes in ionic content. These changes are related to the lipid peroxidation induced following radiation exposure or to sodium pump mechanism. The decline in potassium level induced by irradiation could be explained depending on that hypoxia of blood enhances specific cells (which have  $O_2^-$ -sensitive  $K^+$  channels) in carotid bodies to reduce the  $K^+$  efflux from tissue cells. Reduction in  $K^+$  efflux causes depolarization of the cells which consequently leads to  $Na^+$  influx (Ganong, 1999).

C-reactive protein (CRP) is an exquisitely sensitive systemic marker of inflammation and tissue damage. It is one of acute phase proteins, whose concentrations can change rapidly in response to abnormal events that disturb physiologic homeostasis, including infection, tissue injury and trauma (Clapp *et al.*, 2005). In the present study the significant increase in the level of serum CRP might be attributed to radiation-induced oxidative damage and increases in inflammatory activity (Hayashi *et al.*, 2001). Supplementation of rats with *E. purpurea* has significantly ameliorated serum CRP level returning it to normal. This could be attributed to the role of *E. purpurea* in minimizing radiation-induced oxidative injury as well as to its anti-inflammatory effect (Raso *et al.*, 2002).

The integrity of the immune system depends upon the normal functioning of lymphoid organs so that alterations in the homeostasis of spleen tissues will affect immune responses. The spleen plays an important role in immune functions by proliferating lymphocytes (Witztum, 2002). In the current study, the decrease in lymphocytes might result from oxidative damage in spleen tissues. Furthermore, studies have demonstrated that lymphocytes are considered to be the most sensitive type of blood cells (Wintrobe *et al.*, 1999) and the earliest blood change following whole body irradiation is lymphopenia (Seddek *et al.*, 2000).

In the present study, gamma irradiation results in a significant decrease of total WBCs, lymphocytes, monocytes, neutrophils, basophils and eosinophils. The results are consistent with previous findings that irradiation induced leucopenia (Mishima *et al.*, 2004), and reduces lymphocytes, neutrophils, and monocytes count (Hari *et al.*, 2004). These decreases could be attributed to the high radio sensitivity of haematopoietic tissue (Chew and Park, 2004) and a reduction in the viability of spleen hematopoietic stem cells (Miura *et al.*, 1998). On the other hand, immune cells are particularly sensitive to oxidative stress because their plasma membranes contain a high percentage of polyunsaturated fatty acids (PUFA) (Chew and Park, 2004). So, the decrease in white blood cells differential count recorded in the irradiated rats might be the consequence of radiation-induced lipid peroxidation and damage of their cell membranes.

Treatment with *E. purpurea* significantly increased total WBCs, lymphocytes, monocytes, neutrophils, basophils and eosinophils showing an obvious degree of protection due to the enhancement of the immune system. These results agree with the findings of Barrett, (2003) and Widel *et al.*, (2003), who reported that Echinacea preparations influenced the leukocyte count. Also Di Carlo *et al.*, 2003 reported that the splenic-lymphocytes from mice orally treated with Echinacea for 14 days at a dose level 30 mg/kg/ day were shown to be significantly more resistant to apoptosis. In addition, *E. purpurea* exert a suppressive effect on radiation-induced leucopenia, especially on lymphocytes and monocytes (Mishima *et al.*, 2004). Studies have shown that for patients undergoing radiation and chemotherapy treatments, *E. purpurea*, while boosting the immune system, produced additional white blood cells and stimulated bone marrow production (Rosenthal and Ades, 2001).

The antioxidant activity of *E. purpurea* is mediated through free radical scavenging and transition metal chelating properties (Izzo and Ernst, 2001). The flavonoids present in *E. purpurea* include quercetin, kaempferol, isorhamnetin, anthocyanins and patuletin-3-rutinoside as well as phytomelanin deposits (Kim *et al.*, 2002). The echinacoside and caffeic are antioxidants to which membrane protection is attributed. These are potent scavengers of hydroxyl radicals (OH) and superoxide ( $O_2^-$ ) (Hu and Kitts, 2000), which prevent cellular membrane destruction by oxidation and suppress the drop in white blood cells.

In conclusion, *Echinacea purpurea* is shown to have a radio-protective effect against gamma irradiation by preventing oxidative stress in spleen tissues and modulation of immune responses. Thus supplementation with *Echinacea purpurea* may have a benefit for safe application of radiation technology in medicine and industry.

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