ANTIGENOTOXIC POTENTIAL OF TANACETUM PARTHENIUM LEAF EXTRACT AND SYNTHETIC COMPOUND PARTHENOLIDE AGAINST RADIATION INDUCED MICRONUCLEI FORMATION AND OXIDATIVE STRESS IN SWISS ALBINO MICE

[1]POOJA S, [2]PRASHANTHSHETTY, [3]SUCHETHA KUMARI.N, [4]JAYARAM SHETTY K

[1,2]Central Research Laboratory,[3]Department of Biochemistry,[4]Department of Oncology, K. S. Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India.
E-mail: [1]poojacs77@gmail.com, [2]dprashanthshetty@gmail.com, [3]suchetha.shetty@redifmail.com, [4]jairamshettyk@gmail.com

Abstract -
Background: Radiotherapy is frequently used as a part of cancer treatment to achieve tumor control. However, treatment has been widely used as an effective tool to kill tumor cells; it might produce harmful effects to surrounding healthy tissues. Radiations induce oxidative stress on target tissues, mainly through the generation of reactive oxygen species (ROS) resulting in imbalance of the pro and antioxidants in the cells, in DNA, lipids, and proteins, eventually inducing cell death. Literature review reveals that Tanacetum species have been used as remedies in traditional medicine since ancient times throughout the world.
Objective: To evaluate the antigenotoxic potential of Aqueous and Ethanolic extracts of Tanacetum parthenium (Linn.) and synthetic compound Parthenolide against Electron beam radiation (EBR) induced micronuclei formation and oxidative stress in Swiss Albino mice.
Methodology: Radiation induced micronuclei was performed in the bone marrow of mice according to the method of Hosseinimehret al., 2003. The oxidative stress was carried out to estimate Lipid peroxidation markers (MDA) in the liver homogenate of the mice according to the method of Subash et al., 2011. The experimental animals were orally administered with 100mg/kg b.wt. ethanolic and aqueous extracts of Tanacetum parthenium and 4mg/kg b.wt. Synthetic compound parthenolide once daily for 15 days before exposure to sub-lethal dose (6Gy) of Electron Beam Radiation. Control (C), Radiation Control (RC) and drug control (DC) group was maintained.
Results: The number of Micronucleated Polychromatic Erythrocytes (MnPCE) and Micronucleated Normochromatic Erythrocytes (MnNCE) were decreased in pre treatment groups when compared to radiation control group. Pre treatment was able to reduce Malondialdehyde (MDA) level when compared to radiation control group.
Conclusion: Present findings reveal the antigenotoxic potential of micronuclei formation and reduced lipid peroxidation which may be attributed to scavenging of radiation induced free radicals.

Keywords - Tanacetum parthenium, Electron Beam Radiation, Swiss albino mice, Malondialdehyde and Micronucleus

I. INTRODUCTION

Radiation causes various pathophysiological alterations in living animals, and it causes death at high doses by multiple mechanisms, including direct DNA damage and indirect oxidative stress [1,2]. The search for useful radioprotectors has been an important issue in the field of radiation biology [3]. Ideal radioprotectors should have low toxicity and an extended window of protection [1,4]. As many synthetic compounds have toxic side effects, the natural products have attracted scientific attention in protection against irradiation damage as radioprotectors [4]. Tanacetum parthenium (L.) is an aromatic perennial plant which is basically found in the northern hemisphere. The plant is also been cultured in gardens. It is commonly known as feverfew. T. parthenium has long been used to treat headache, stomach-ache, fever, toothache, insect bite, menstrual irregularities and as anti-bacterial by the herbal experts. Compounds found in T. parthenium have metal chelating and free radical scavenging property. Many researchers have stated that bioactive component present in T. parthenium corresponding for the pharmacological functionality is parthenolide [5].

II. MATERIAL AND METHODS

Animals

The use of animals was approved by the Institutional Animal Ethics Committee of Nitte University, India (Ref. KSHEMA/IAEC/24/2014 dated 07-11-2014). Healthy adult Swiss albino female mice, 6-8 weeks old with average body weight (b.w) of 20 to 30g were used for the study. The animals were selected randomly and numbered with ear tag and housed in autoclaved polypropylene boxes with rice husk bedding and steel wire top. The temperature, light and humidity were maintained at 27 ± 2 ºC, 12 h dark/light and 55±7%.

Plant material

The entire experiment was carried out at the Central Research Laboratory (CRL), Nitte University, Mangalore, India. The Ethanolic and Aqueous leaves extract of T. Parthenium was obtained from Organic Inc China. The synthetic compound parthenolide

(98% min; HPLC graded) was obtained from Shanghai Better BioChem Co., Limited China.

Mode of administration and treatment

Swiss Albino mice were divided into the following groups with 6 animals in each group (n = 6): Group I (Control) was administered with distilled water, Group II (Irradiated group) were given distilled water before 6-Gy irradiation, Group III (Treated group) were treated with 100 mg/kg b.w of ethanolic T. parthenium extract, Group IV (Treated group) animals were treated with 100 mg/kg b.w of aqueous T. parthenium extract and Group V (Treated group) were treated with 4 mg/kg b.w of synthetic parthenolide. The treated group started receiving treatment each day by oral gavage for fifteen days prior to radiation.

All animals were exposed to a single dose of radiation (6 Gy) on the 15th day. Then, 24 hr after irradiation, the animals were sacrificed by cervical dislocation. Femur bone and liver samples were collected.

Irradiation of animals

On the 15th day, one hour after the administration of drug the mice were placed in ventilated Perspex boxes with a dimension 3x6 cms and subjected to whole body radiation (energy linear accelerator unit) with a sub-lethal dose of 6 Gy of electron beam radiation at a dose rate of 3 Gy/min, with a source to target distance of 100cms. This was carried out in the department of Oncology, K.S.Hegde Charitable Hospital, Mangalore, Karnataka, India.

Micronucleus Assay

The frequency of micronucleated polychromatic erythrocytes in femoral bone marrow was evaluated according to the procedure described by Hosseinimehr [4] and Schmid [6] with some modifications. Bone marrow from the femur was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of Bovine serum albumin. Smears were made on pre-cleaned, pre-cooled, dry slides, air dried and fixed in absolute methanol for 30 min and stained with Geimsa. The slides were observed under a light microscope to seek micronuclei in polychromatic erythrocytes (PCEs). A total of 1000 erythrocytes were scored in each animal. The number of micronuclei in PCEs was expressed as a percentage value to depict the MN frequency. The frequencies of MnPCE and normal PCE between treated and control groups are compared and the results were expressed as mean ± standard error and analyzed statistically using Tukey HSD test.

Estimation of Lipid Peroxidation in liver Homogenate:

LPO was measured using the method of Subash [8]. Malondialdehyde (MDA) formed from the breakdown of poly-unsaturated fatty acids which serves as a convenient index for determining the extent of the peroxidation reaction. Peroxidation of lipids generates MDA, which reacts with TBA to give a red colour to the solution with absorbance at 535 nm. Results were expressed as mean ± standard error and analyzed statistically using Tukey HSD test.

III. RESULTS AND DISCUSSION

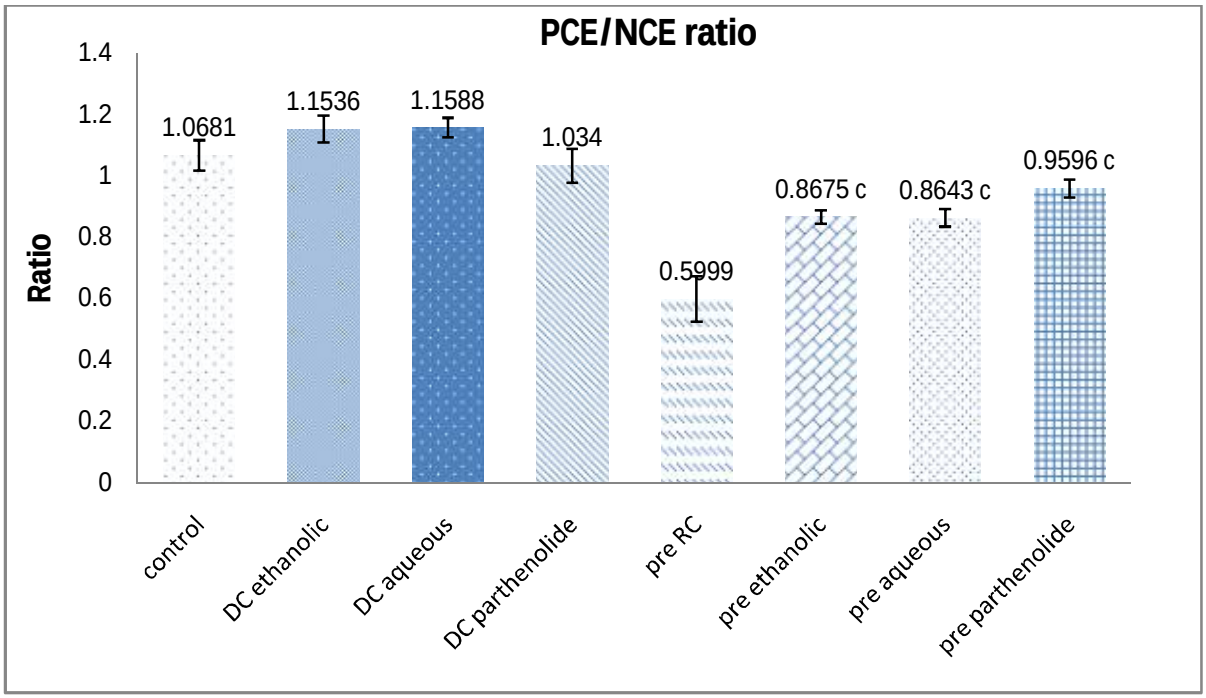


Figure1: PCE/NCE ratio of Aqueous and Ethanolic extracts of Tanacetum parthenium (Linn.) and Parthenolide among different drug and pre treatment groups under study.

Values are mean ± SEM from 6 mice/ group. Significant levels are, a = P < 0.0001compared to control; b = P < 0.001 compared to irradtion control; c = P < 0.001 compared to treatment group + irradiation group; No symbol = non-significant.

Effect of the ethanolic and aqueous extracts of T.parthenium and parthenolide on bone marrow nucleated cells: The number of bone marrow cells (Figure 1) was significantly lower in the irradiation-only group than in the normal control group. In the irradiation groups that had been pre-treated with the extracts and parthenolide the cells were significantly higher than the irradiation-only group ($p < 0.001$ for each treatment group). Micronuclei are the known cytogenetic end points generally used for evaluating genotoxicity. Micronuclei are formed in the cytoplasm of the cell due to chromosome damage by external factors such as radiation [8]. The pretreatment mice were effective in the induction of micronuclei. In the present study, an elevation in the ratio of PCE/NCE was observed after irradiation in both control and experimental groups. Phytochemicals and antioxidant might provide a variable degree of radioprotection. Therefore it is possible that pretreatment with extracts of medicinal plants having antioxidant activity [9] could protect the biological system against oxidative stress.

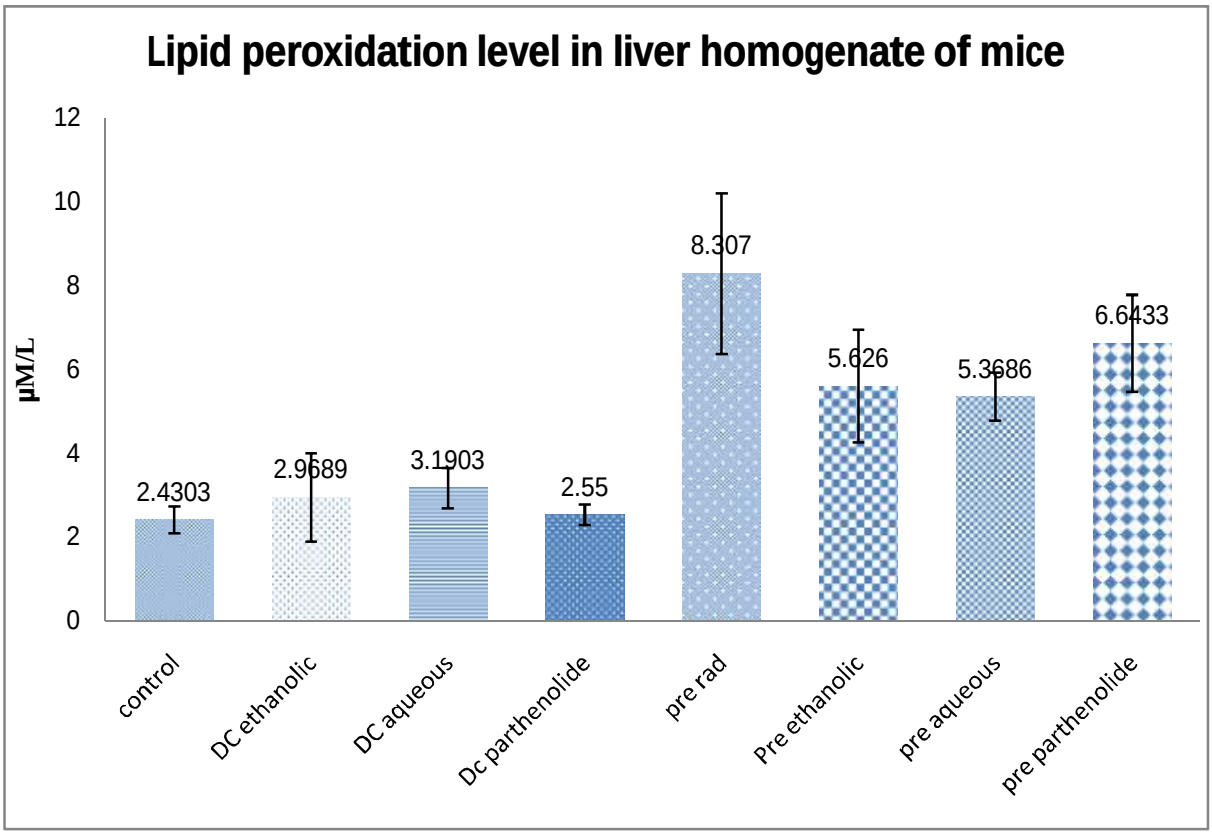


Figure 2: Mean and standard error of the Lipid peroxidation level in Liver homogenate of Aqueous and Ethanolic extracts of Tanacetum parthenium and Parthenolide among different drug and pre treatment groups under study.

In the present study ethanolic, aqueous extracts of T.parthenium and synthetic parthenolide alone did not significantly alter the LPO in unirradiated animals, but ethanolic and aqueous extracts of T.parthenium and synthetic parthenolide pre-treatment significantly lowered the radiation-induced LPO in terms of MDA (figure 2). Therefore, inhibition of LPO by extract and synthetic compound is significant in protecting the cells from radiation-induced damage.

The potential of extracts of T.parthenium and synthetic parthenolide in mitigating radiation-induced oxidative stress is remarkable. Hence this study clearly showed the free radical scavenging activity [9] indicating that it may have its potential as a radioprotective agent.

In conclusion, the results showed that the extracts possess antigenotoxic phytoconstituents with antioxidant activity. These compounds could reduce the oxidative stress by LPO markers and genotoxicity induced radiation in bone marrow cells. It might also due to the influence of the extract on xenobiotic metabolizing enzymes reducing the extent of lipid peroxidation and upregulating antioxidant defenses. From the foregoing review it can be summarized that there are several novel approaches to radioprotection. As we have an enormous heritage of vast natural medicinal resources it is worth exploring the

possibility of developing efficient, economically viable and clinically acceptable radioprotection for human application from these resources.

ACKNOWLEDGEMENTS

The authors would like to thank the Board of Research in Nuclear Science (BRNS), Govt. of India for providing the funding for the project and Nitte University for providing the laboratory facilities to carry out the work. The authors also thank all the colleagues of Nitte University Central Research Laboratory (CRL) for their support.

REFERENCES

[1] M.Karbownik, R.J. Reiter, W. Qi, J.J.Garcia, D.X. Tan, L.C.Manchester, Vijayalaxmi, "Protective effects of melatonin against oxidation of guanine bases in DNA and decreased microsomal membrane fluidity in rat liver induced by whole body ionizing radiation," Mol Cell Bio chem., 211, pp.137-144, 2000.

[2] Y.Miura, "Oxidative stress, radiation-adaptive responses, and aging," J Radiat Res, 45, pp.357-372, 2004.

[3] C.K.Nair, D.K.Parida, T.Nomura, "Radio protectors in radiotherapy," J Radiat Res, 42, pp.21-37, 2001.

[4] S.J.Hosseinimehr, "Trends in the development of radioprotective agents,"Drug Discov Today," 12, pp.794-805, 2007.

[5] D.V.C.Awang, B.A.Dawson, D.G.Kindack, C.W.Crompton and S.Heptinstall, "Parthenolide Content of Feverfew (Tanacetum parthenium) Assessed by HPLC and 1H-NMR Spectroscopy," Jour. Nat. Prod, 54(6), pp.1516-21, 1993.

[6] W. Schmid, "The micronucleus test," Mutation Res, 31(19), pp. 9–15, 1975.

[7] V.K. Subash, G.Saritha, V.Chandra Sekar, "Oxidative Stress and antioxidant status in patients with acute myocardial infarction in MGM Hospital Warangal(A.P), "International Journal of Pharmacy Practice & Drug Research, 1(1), pp.1-7, 2011.

[8] C.Demirel, S.Kilciksiz, O.I.Ay, S.Gurgul, M.E.Ay, N.Erdal, "Effect of N-acetylcysteine on radiation induced genotoxicity and cytotoxicity in rat bone mar- row," J Radiat Res, 50, pp.43–50, 2009.

[9] S.Prashanth, S.Pooja, K.N.Suchetha, Priya, V.Vidya. "Radical scavenging and antioxidant activities of ethanolic and aqueous extract from the leaves of feverfew (Tanacetum parthenium L.) and a synthetic compound parthenolide," Journal of Pharmacognosy and Phytochemistry, 4(1), pp. 223-227, 2015.

★★★