Research Article

BRJ
Biomedical Research Journal

Oral Feeding With Arachidonic Acid (AA) and Docosahexanoic Acid (DHA) Help in Better Recovery of Haematopoiesis in Sub-lethally Irradiated Mice

Kedar Limbkar, Vaijayanti Kale and Lalita Limaye*

Stem Cell Laboratory, National Centre for Cell Science, University of Pune Campus, Pune – 411007, India

Haematopoiesis is severely hampered after exposure to ionizing radiations. Role of polyunsaturated fatty acids (PUFAs) during embryonic development as well as during various physiological processes is well established. However, few studies on their effect on haematopoiesis are reported. Hence, we studied the effect of oral administration of PUFAs-AA/DHA on haematopoiesis of sub-lethally irradiated mice. To determine the optimal dose for haematopoiesis, non-irradiated healthy mice were orally fed with different doses of AA/DHA daily for ten days. Additionally, mice were sub lethally irradiated and kept for ten days on normal diet. Further, sub-lethally irradiated mice were orally fed with optimal dose of AA/DHA for ten days. Mice from the experiments were sacrificed after ten days and their bone marrow cells were harvested and analyzed for their total nucleated cell (TNC) count, side population (SP) and $lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$(LSK) phenotype. Peripheral blood collected from this set of mice was subjected to hemogram analysis. Daily dose of 8 mg AA/DHA for ten days was assessed as optimal for enhancing BM-MNCs and primitive HSCs in non-irradiated mice. Significant depletion in BM-MNCs, SP and LSK cells was observed in sub lethally irradiated mice compared to un-irradiated control mice. Feeding with DHA or AA in sub lethally irradiated mice showed significantly higher number of BM-MNCs and increased percentage of SP and LSK cells, suggesting that DHA and AA resulted in better recovery of hematopoietically compromised mice. The data indicated that DHA or AA may serve as useful dietary supplements in patients exposed to irradiation.

INTRODUCTION

Exposure to ionizing radiations is common in the modern age as they are widely used in research, diagnosis, manufacturing and construction (Brenner *et al.,* 2007). Ionizing radiation is a common modality of treatment of cancer patients. Haematopoiesis maintains blood cell lineages at constant level. Bone marrow provides a favorable microenvironment for hematopoietic stem cells, enabling repopulation, differentiation and migration, and also regulates generation of blood cells (Shen *et al.,* 2010). The extremely proliferative

Key words: HSCs, haematopoiesis, radiation injury, docosahexanoic acid, arachidonic acid, recovery.
***Corresponding Author:** Lalita Limaye, Stem Cell Laboratory, National Centre for Cell Science, NCCS complex, University of Pune Campus, Ganeshkhind, Pune – 411007, Maharashtra, India.
Email: lslimaye@nccs.res.in

property of the HSCs (Ogawa M., 1993) required to maintain homeostasis makes them highly radiosensitive (Chinsoo *et al.,* 1998; Chitteti *et al.,* 2011; Till *et al.,* 1964). A radiation dose of 2-8 Gy may create significant damage to the bone marrow causing the hematopoietic syndrome of the acute radiation syndrome (HS-ARS), characterized by life-threatening lymphocytopenia, neutropenia, and thrombocytopenia, and possible death due to infection and/or bleeding (Anno *et al.,* 1989; Coleman *et al.,* 2001; Simopoulos AP, 2002).

Diet plays a key role in normal functioning and development. ω-3 (n-3) and ω-6 (n-6) polyunsaturated fatty acids (PUFAs) are important structural and functional components of cell membrane phospholipids. These form the essential fatty acids, as they cannot be synthesized in the human body and must be obtained from diet (Gebauer *et al.,* 2006). As essential nutrients obtained only through dietary intake, their tissue content in individuals can vary, but may be modified through dietary intervention. The beneficial effects of DHA and AA are observed in humans and animal models of diabetes, obesity, cancer, hypertension, autoimmune disorders, mental health, and cardiovascular diseases, etc. They play an important role in embryonic development, development of vision and neuronal development. The metabolites play key role in cell signaling, and thereby modulate various physiological and pathological processes (Belluzzi *et al.,* 1996; Ismail HM, 2005; Shannon *et al.,* 2007; Simopoulos, 2009). PUFAs also get incorporated in membrane lipid raft, consequently altering the membrane composition (Turk *et al.,* 2013). These lipid rafts have important role in embryonic stem cell self-renewal (Lee *et al.,* 2010). The metabolism of AA/DHA is depicted in the flow chart below (Fig. 1). AA is broken down to either leukotrienes, prostaglandins or eicosatetraenoic acids by lipoxygenases, cyclooxygenases and cytochrome P450, respectively. Similarly, DHA is metabolized through lipoxygenases to resolvins.

The effect of PUFAs on haematopoiesis is complex, since these fatty acids are processed into leukotrienes, eicosanoids and prostaglandins, which independently affect haematopoiesis. Several reports suggest that the PUFAs act on human marrow myelopoiesis and erythropoiesis as evidenced by the growth of committed progenitors (CFU-GM and BFU-E) in

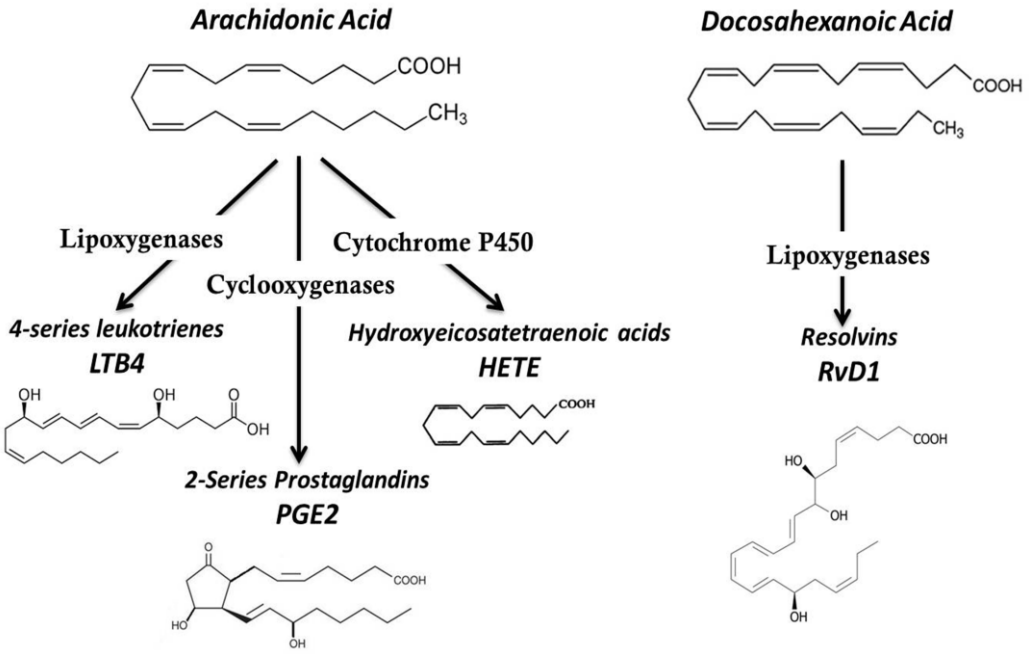

Figure 1: Flow chart showing metabolism of AA/DHA.

vitro (Dupuis *et al.,* 1997). It has been reported that diet rich in n-3 PUFAs relative to the proportion of n-6 PUFAs, affects myelopoiesis by reducing total myeloid progenitor cell frequency and promotes differentiation of specific progenitor cell types in the bone marrow of mice (Verny *et al.,* 2009). Besides, AA and DHA influences megakaryopoiesis and thrombopoiesis *in vitro* (Shabrani *et al.,* 2012; Siddqui *et al.,* 2011).

Thus, our hypothesis was 'whether oral feeding of PUFAs in hematopoietically compromised mice, enhances haematopoiesis in mice'. In the present study, we demonstrate that feeding sub-lethally irradiated mice with DHA or AA orally for ten days enhances the bone marrow cell count and increases haematopoiesis.

MATERIALS AND METHODS

Mice

Protocols used in the animal experimentation were approved by the Institutional Animal Ethics Committee (IAEC). C57BL/6 mice (6–8 weeks old, females) were used for the feeding experiments.

Nutraceuticals

Docosahexanoic acid (> 99% Pure) and Arachidonic acid (> 99% Pure) were procured from NuChek Prep (Elysian, USA).

Oral Feeding of Mice With Nutraceuticals

The following protocol was followed: 1) Mice were fed various doses of AA/DHA: 2, 4, 8 and 16 mg. Control mice were fed with PBS (vehicle control). 2) Control and test mice were subjected to dose of 4.5 Gy irradiation using ^{60}Co Gamma Chamber (BRIT, Mumbai, India) and kept on normal diet for ten days. Non-irradiated mice were kept as control. 3) Control mice and test mice were sub-lethally irradiated as described above. Test mice in addition to normal solid feed were fed 8 mg AA/DHA daily through oral feeding gavage in separate sets for ten days. Mice fed with PBS (henceforth will be referred as unfed) were used as control.

Harvesting and Processing of BM and PBL

Mice from all experiments were sacrificed after ten days and their bone marrow mononuclear cells (BM-MNCs) were harvested by flushing tibia and femur bones with 21G syringe. Total nucleated cells (TNCs) were counted manually using hemocytometer after mixing them with Turk's solution containing crystal violet and acetic acid. They were further subjected to flow cytometry analysis of HSCs like SP and LSK analysis.

Blood was collected from mice that were irradiated and then fed with PBS/AA/DHA and was subjected to hemogram analysis using automated blood cell counter.

Side Population (SP) Analysis

SP analysis was performed as described by Eaker *et al*. (Eaker *et al*., 2004). Briefly, 10^6 BM MNCs of fed or unfed mice were stained with 5 µg Hoechst 33342(Sigma), with or without 50 µM Verapamil (Sigma Aldrich, St Louis, USA), for 90 min at 37°C. The cells were stained with 50 µM Propidium Iodide (PI) for detecting dead cells. The cells were analyzed on a flow cytometer (FACS ARIA III SORP, Becton Dickinson) using UV laser.

Phenotypic Analysis

LSK analysis was performed as per Uchida *et al.* (Uchida *et al.*, 1992). Briefly, 10^6 BM MNCs were suspended in IMDM containing 20% FBS. The cells were washed and suspended in PBS containing 0.1% BSA and 0.1% sodium azide, and stained with c-Kit CD117-PE-Cy7, CD45.2-PB, lineage marker cocktail (CD3e, CD11b, CD45R/B220,

Ly-76, Ly-6G, and Ly-6C)-APC, Sca-1/Ly-6A/E-PE (BD Bioscience, San Diego, USA), at 4°C for 45 min with frequent mixing. The cells were washed with PBS and fixed in 1% buffered paraformaldehyde. Appropriate isotype controls were used. Fifty thousand events in the lineage negative gate were acquired for each sample (FACS Canto II; BD Bioscience, San Diego, USA). The flow cytometry data was analyzed using FACS Diva™ (BD Bioscience) software. c-Kit and Sca-1 double positive population was gated in lineage negative cells to get LSK population.

Statistical Analysis

Statistical analysis was done using Sigma Plot 11 (Jandel Scientific Corporation, San Rafael, California, USA) software using One Way RM-ANOVA. The mean and standard deviation obtained was plotted for the various assays. The data was considered significant if $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). Graphs were plotted using the same software.

RESULTS

Optimal Dose of AA/DHA for Haematopoiesis in Mice

To determine optimal dose of AA or DHA for haematopoiesis, mice were fed for ten days with 2, 4, 8 or 16 mg of AA or DHA, respectively. PBS fed (Unfed) mice were kept as sham control. Mice were sacrificed after 10 days of feeding and their bone marrow mononuclear cells (BM-MNCs) were subjected to total nucleated cell (TNC) count, SP and LSK analysis. As shown in the Fig. 2A, BM MNCs of mice fed with 8 mg AA /DHA showed significantly higher number of TNCs as compared to control mice (PBS fed), indicating 8 mg as the optimal dose. Side population cells are known to give prolonged multi lineage haematopoiesis since they harbor long-term repopulating stem cells. Fig. 2B, shows representative FACS profile of fed and unfed mice for SP cells. Specificity of SP phenotype was confirmed by addition of Verapamil known to abolish SP profile. Cumulative data from five mice indicate that oral dose of 8 mg of AA/ DHA was optimal for stimulating side population (Fig. 2C). LSK cells are known to be primitive stem cells. As observed in Fig. 2E, marrow cells of the AA-fed and DHA fed mice showed higher percentage of LSK cells as compared to the controls. Representative FACS profile is depicted in Fig. 2D. Thus the data show that oral dose of 8 mg of AA or DHA enhances haematopoiesis in mice.

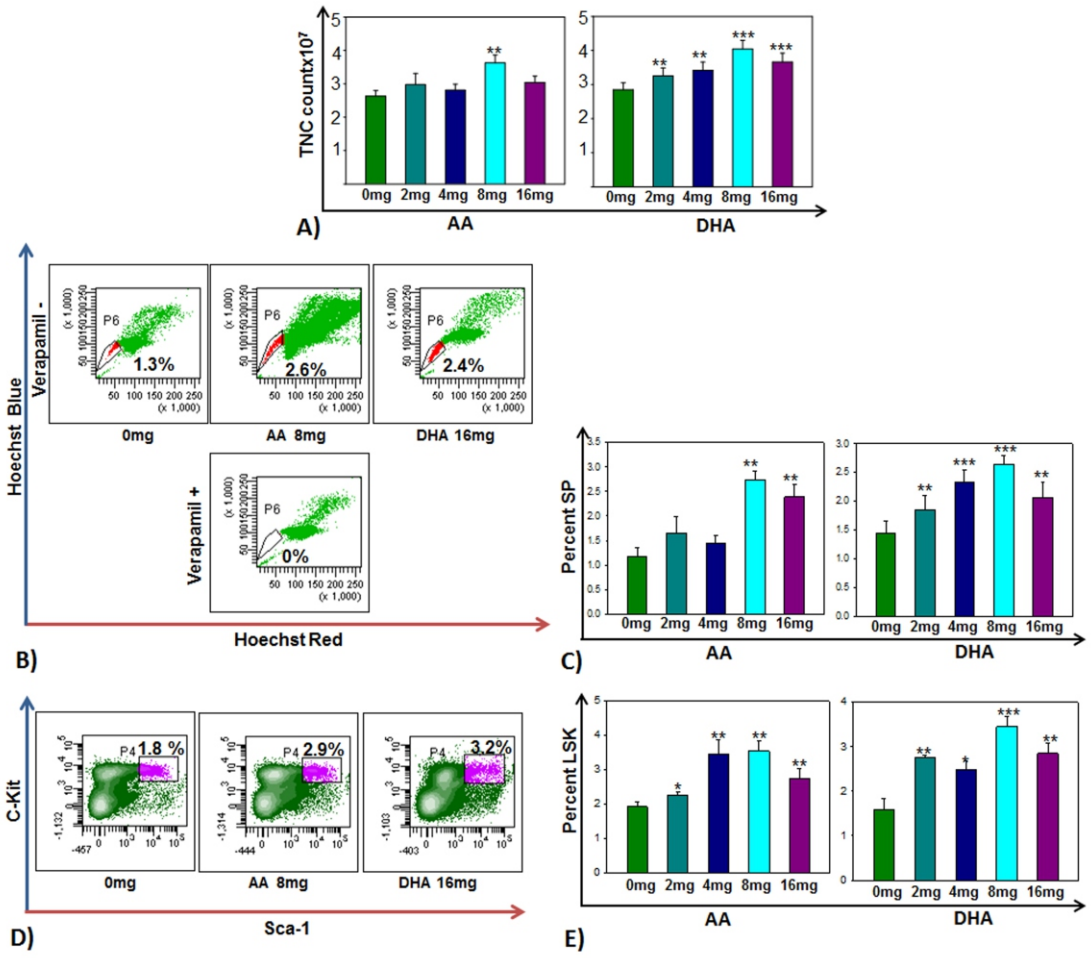

Figure 2. Dose optimization for AA/DHA. To determine optimal dose for haematopoiesis, mice were fed with different doses of AA and DHA daily for ten days. Their BM MNCs were harvested and subjected to various assays for haematopoiesis. Cumulative data from 4 samples clearly shows that dose of 8mg AA/DHA significantly enhanced (A) TNC count; (C) SP; (E) LSK population compared to control. Representative flow cytometry profile from Fig., (B) and (D), depicts the same. N = 5; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Sub-lethal Irradiation Depletes Bone Marrow Cells and Hscs in Mice

To study the effect of irradiation on haematopoiesis, mice were given sub lethal dose (4.5 Gy) of irradiation; healthy, non-irradiated mice were kept as control. Mice were kept untreated for 10 days and sacrificed after 10 days .The bone marrow cells were harvested and analyzed for TNC count, SP cells and LSK cells. Fig. 3A shows that sub lethal irradiation significantly depleted total nucleated cells in mice. Flow cytometry profile (Fig. 3B) and cumulative statistical data in Fig. 3C shows more than two fold reduction in side population cells of irradiated mice. Sub lethal dose of gamma irradiation causes

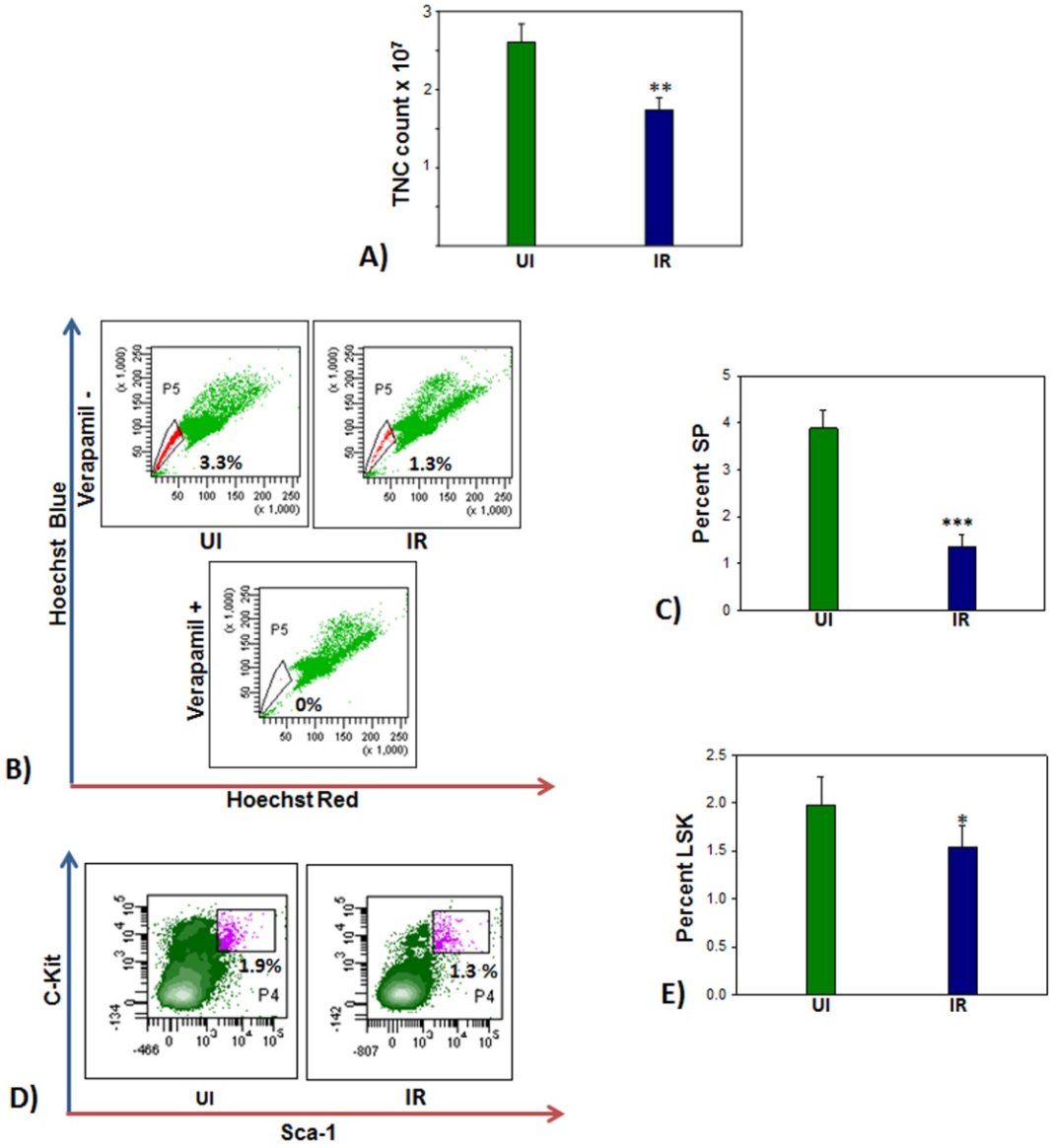

Figure 3. Irradiation hampers haematopoiesis in mice. Mice were sub lethally irradiated and were kept for ten days without any treatment. Non-irradiated mice were kept as control. Mice were sacrificed and their BM MNCs were tested for hematopoiesis. Data clearly shows sub lethal dose of irradiation caused significant decrease in the (A) TNC count, (C) SP and (E) LSK cells in mice. Representative flow cytometry profile of (B) SP and (D) LSK depicts the same. N = 4; *p < 0.05, **p < 0.01, ***p < 0.001.

hematopoietic ablation in mice. Irradiation decreased primitive stem cells as observed by decreased percent LSK (Fig. 3E). Representative flow cytometry profile is depicted in Fig. 3D.

Feeding AA or DHA to Sub-lethally Irradiated Mice Restores Haematopoiesis

Sub-lethally irradiated mice were fed with AA or DHA for ten days. Mice were sacrificed and bone marrow (BM) cells

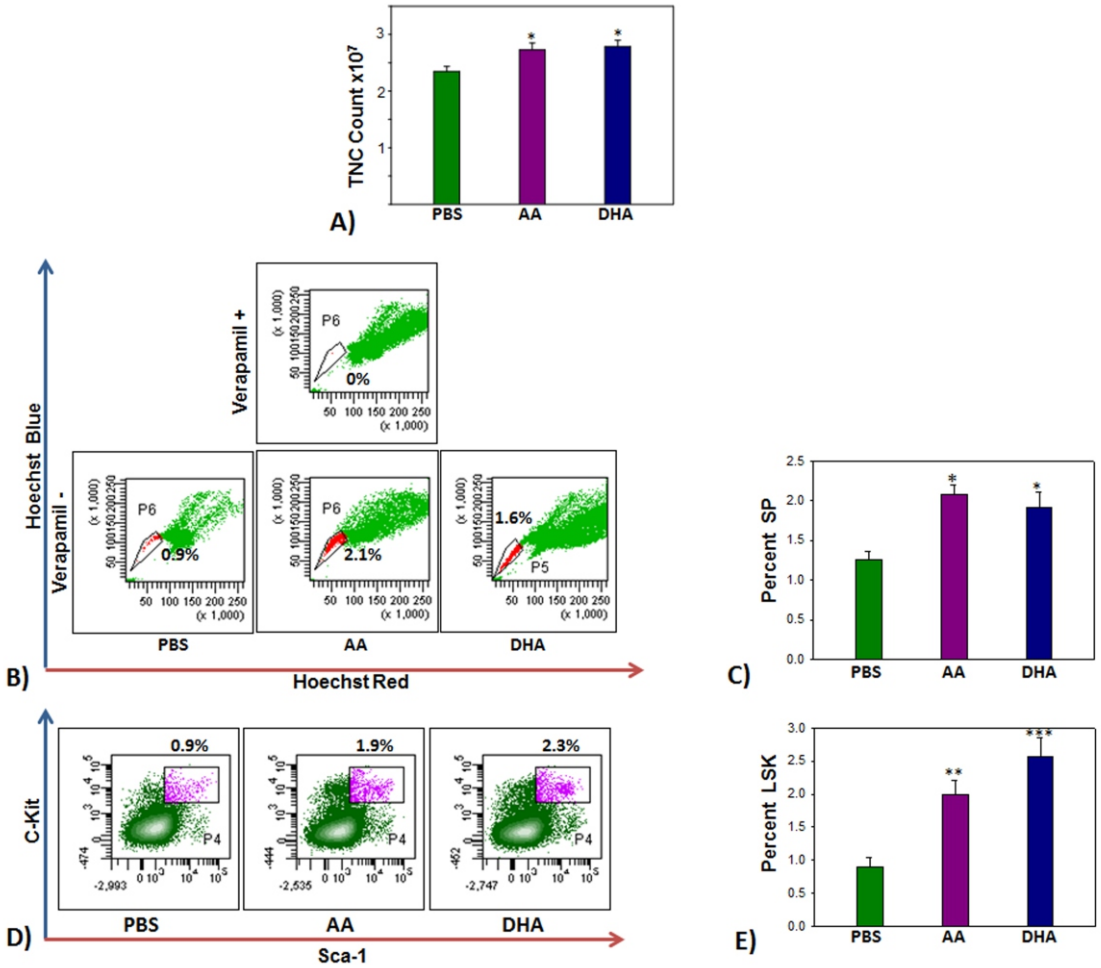

Figure 4. Feeding of AA/DHA restores haematopoiesis in irradiated mice. Mice were given sub lethal dose (4.5 Gy) of irradiation and were fed daily for 10 days with either PBS (control) or 8mg AA /DHA. Mice were sacrificed after 10 days and their BM MNCs were analyzed for hematopoiesis. Irradiated mice, when fed with AA or DHA, showed significant increase in their (A) TNC count, (C) SP and (E) LSK percentage. Flow cytometry profile of one representative sample (B) and (D) also suggests the same. N = 5; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

and peripheral blood (PBL) cells were harvested. Total nucleated cells were increased in BM MNCs of AA or DHA fed mice, as compared to control mice (Fig. 4A). AA/DHA stimulated long term repopulating cells. As shown in Fig. 4B, increased percentage of SP cells was observed in AA (2.1%) and DHA fed mice (1.6%). Cumulative data clearly indicates that AA and DHA caused significant enhancement in the percentage SP in the bone marrow (Fig. 4C). AA and DHA stimulated primitive stem cells. Fig. 4D shows increased percentage of LSK cells in AA fed (1.9%) and DHA fed (2.3%) mice. Significantly increased number of LSK cells were observed in bone marrow of fed mice (Fig. 4E).

Peripheral blood cells of unfed and

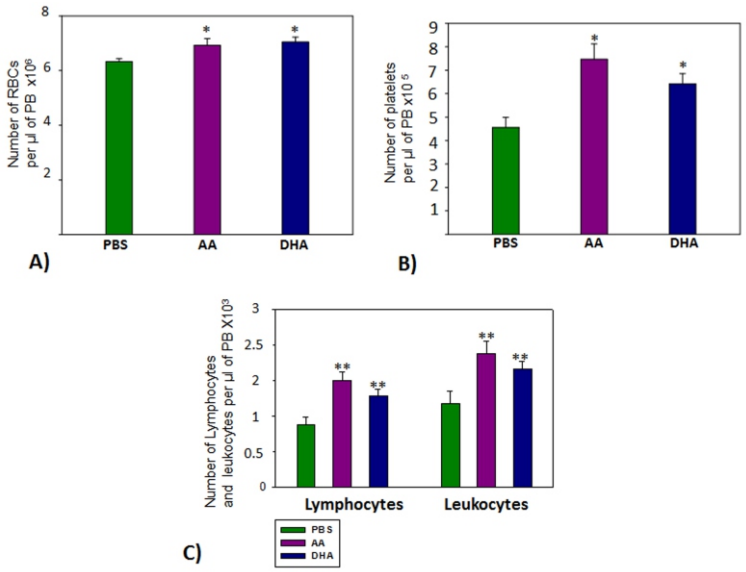

Fig. 5. AA/DHA increased RBC, Platelet and Leukocyte production. Peripheral blood of sub lethally irradiated and fed/unfed mice was subjected to automated blood count analysis. Significant enhancement in (A) RBC number and (B) platelet count was observed in AA/DHA fed mice. AA/DHA feeding also caused significant increase in (C) leukocytes, especially lymphocytes compared to control. N = 5; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

fed mice of this set of experiment were subjected to hemogram analysis. It was observed that feeding of AA and DHA resulted in increased RBC (Fig. 5A) and platelet count (Fig. 5B) in mice. AA and DHA significantly increased leucocytes especially lymphocytes in the peripheral blood of mice (Fig. 5C).

Thus, the data suggests that oral administration of AA or DHA to normal healthy mice stimulates the haematopoiesis. When sub lethally irradiated mice were fed with DHA/AA, it stimulated their long-term repopulating cells, primitive HSCs and promoted enhancement of erythropoiesis and thrombopoiesis.

DISCUSSION

In the present study, we have made a systematic attempt to examine the effect of PUFAs - AA (n6 PUFA) and DHA (n3PUFA) on haematopoiesis of sub lethally irradiated mice. We optimized the dose of the two PUFAs for maximal stimulation of haematopoiesis. Daily oral dose of 8 mg of AA/DHA was beneficial. Our results are accordance with earlier studies. Hoggatt *et al*. (2009) who reported that short-term ex vivo exposure of HSCs to PGE2 -a prostaglandin, derived from AA, enhances their homing, survival and proliferation, resulting in increased long-term repopulating cell (LTRC) and competitive repopulating unit (CRU) frequency. However, the

authors used PGE2 and studied *in vitro* effect on HSCs, whereas we report effect of *in vivo* feeding of purified PUFAs on haematopoiesis. Several studies suggest role of n3PUFAs or n6 PUFAs or their metabolites in stem cell proliferation (Beltz *et al.,* 2007; He *et al.,* 2009; Kawakita *et al.,*2006; Kim *et al.,* 2009; Thangavelu *et al.,* 2007). Our systematic study indicates a direct correlation between oral feeding of AA/DHA and stimulation of haematopoiesis in mice.

Further, we examined the effect of sub-lethal dose of irradiation on haematopoiesis in mice. A reduction in TNC count followed by drastic reduction in SP and LSK cells are hallmark effects of irradiation. Depletion in TNC count may be attributed to hampered self-renewal of HSCs, confirmed by significant reduction in SP cells and LSK percentage. Our data is consistent with earlier reports suggesting that ionizing radiation hampers HSC self-renewal and acute radiation causes BM failure (Hu *et al.,* 2010; Lorrimore *et al.,* 2003; Weiss *et al.,* 2000).

We examined the effect of optimized daily dose of 8 mg of AA/DHA for ten days on sub-lethally irradiated mice and checked their effect on haematopoiesis. We observed significant increase in TNC count, SP cells and LSK cells. Enhancement in haematopoiesis may be because of protective role of PUFAs from radiation injury. Our data are in line with study done by Hoggatt *et al*. (2013), reporting that subcutaneous administration of PGE2 analog, to mice after irradiation, increased their survival by enhancing white blood cells (WBC), polymorphonuclear leukocytes (PMN) and platelets (PLT) over a 30 day period indicating enhanced haematopoietic recovery in mice after irradiation. Gómez de Segura *et al*. (2004) have reported that supplementing the diet with DHA prevented the negative action of 5-FU on mucosal morphometry in rats. Umegaki *et al*. (1997) noted that by feeding mice a diet containing oleic acid before X-ray exposure, experienced greater degrees of immunosuppression (53% and 69%, respectively) than did those consuming diets containing eicosapentaenoic acid alone or in combination with docosahexaenoic acid (DHA) (4% and 24%, respectively). We also observed enhancement in erythropoiesis, thrombopoiesis and leukocytes in PBL of irradiated mice fed with AA/DHA. No significant change in the number of eosinophils, neutrophils, monocytes and granulocytes was observed in PBL of fed

mice (data not shown) suggesting that oral administration of AA/DHA in mice is not causing any lineage bias. Recent study of Xia *et al*. (2015) showed that fish oil-rich diet promotes hematopoiesis in the bone marrow and spleen of mice by increasing TNC count, WBC count and LSK cells in part via the activity of MMP12. However in the study fish oil was mixed with the solid diet of mice. Whereas, we orally administered the defined amount of pure AA/DHA to mice.

Thus, our results demonstrate that oral feeding of AA or DHA enhances haematopoiesis in irradiated mice, and helps in partial recovery from hematopoietic injury. Further studies such as investigating radio-protectant effect of AA/DHA in the context of their ability to quench ROX species and studying mechanism of action of PUFAs will add to their therapeutic application. Dietary interventions of AA or DHA may also enhance stem cell recovery from radiation injury, and hence indicated as an adjunct supplement to radiotherapy, for better recovery of haematopoiesis.

ACKNOWLEDGEMENT

The authors thank Dr. Mande, Director, NCCS, for support; experimental animal facility (EAF) of NCCS for care and maintenance of mice; FACS facility for acquisition of samples, Dr. R. L. Marathe for analysis of PBL samples, Mrs. Nikhat Khan for technical help. We thank Department of Biotechnology (DBT), Government of India, New Delhi for project grant (LL/BT/PR-4930/2013-15), and Indian Council of Medical Research (ICMR), Government of India, New Delhi, for doctoral fellowship of KRL.

CONFLICT OF INTEREST

No conflict of interest.

REFERENCES

Anno GH, Baum SJ, Withers HR, Young RW. Symptomatology of acute radiation effects in humansafter exposure to doses of 0.5–30 Gy. *Health Phys.* 1989;56:821–838.

Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M. Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *N Engl J Med.* 1996; 334:1557–1560.

Beltz BS, Tlusty MF, Benton JL, Sandeman DC. Omega-3 fatty acids upregulate adult neurogenesis. *Neurosci Lett* 2007;415:154–158.

Brenner DJ, Hall EJ. Computed tomography--an increasing source of radiation exposure. *N Engl J Med.* 2007;357(22):2277–2284.

Chinsoo CL, Glatstein E. Radiation Injury. *In*: Fauci AS, Braunwald E, Isselbacher KL,

editors. *Harrison's Principles of Internal Medicine*. McGraw-Hill; New York: 1998. 2548–2565.

Chitteti BR, Kacena MA, Srour EF. Phenotypic characterization of hematopoietic stem cells. In: Broxmeyer HA, editor. *Cord Blood: Biology, Transplantation, Banking, and Regulation.* AABB Press; Bethesda: 2011. 75–85.

Coleman CN, Blakely WF, Fike JR, MacVittie TJ, Metting NF, Mitchell JB, *et al.,* Molecular and cellular biology of moderate-dose (1-10 Gy) radiation and potential mechanisms of radiation protection: report of a workshop at Bethesda, Maryland, December 17-18, 2001. *Radiat Res.* 2003;159(6):812–834.

Dupuis F, Desplat V, Praloran V, Denizot Y. Effects of lipidic mediators on the growth of human myeloid and erythroid marrow progenitors. *J Lipid Mediat Cell Signal.* 1997;16(3):117–125.

Eaker SS, Hawley TS, Ramezani A, Hawley RG. Detection and enrichment of hematopoietic stem cells by side population phenotype. *Methods Mol Biol.* 2004; 263:161–180.

Gebauer Sk, Psota TL, Harris, WS, Kris-Etherton PM. N-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits, *Am J Clin Nutr*; 2006, 83 (Suppl.): 1526–1535.

Gómez de Segura IA, Valderrábano S, Vázquez I, Vallejo-Cremades MT, Gómez-García L, Sánchez M, de Miguel E. Protective effects of dietary enrichment with docosahexaenoic acid plus protein in 5-fluorouracil-induced intestinal injury in the rat. *Eur J Gastroenterol Hepatol*. 2004;16(5):479–485.

He CW, Qu XY, Cui LB, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. *Proc Natl Acad Sci USA* 2009; 106:11370–11375.

Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 2009; 113:5444–5455.

Hoggatt J, Singh P, Stilger KN, Plett PA, Sampson CH, Chua HL, *et al*. Recovery from hematopoietic injury by modulating prostaglandin E (2) signaling post-irradiation. *Blood Cells Mol Dis* 2013; 50:147–153.

Hu KX, Sun QY, Guo M, Ai HS. The radiation protection and therapy effects of mesenchymal stem cells in mice with acute radiation injury. *Br J Radiol.* 2010;83(985):52–58.

Ismail HM. The role of omega-3 fatty acids in cardiac protection: an overview. *Front Biosci.* 2005; 10:1079–1088.

Kawakita E, Hashimoto M, Shido O. Docosahexaenoic acid promotes neurogenesis in vitro and in vivo. *Neuroscience* 2006; 139: 991–997.

Kim MH, Kim MO, Kim YH, Kim JS, Han HJ. Linoleic acid induces mouse embryonic stem cell proliferation via Ca21/PKC, PI3K/Akt, and MAPKs. *Cell Physiol Biochem* 2009;23:53–64.

Lee MY, Ryu JM, Lee SH, Park JH, Han HJ. Lipid rafts play an important role for maintenance of embryonic stem cell self-renewal. *J Lipid Res* 2010; 51:2082–2089.

Lorimore SA, Coates PJ, Wright EG. Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of

exposure to ionizing radiation. *Oncogene*. 2003; 13;22(45):7058–7069.

Ogawa M. Differentiation and proliferation of hematopoietic stem cells. *Blood*. 1993 1; 81(11):2844–2853.

Verny ME, Hardman WE, Sollars VE. Omega 3 fatty acids reduce myeloid progenitor cell frequency in the bone marrow of mice and promote progenitor cell differentiation, *Lipids in Health and Disease*; 2009; 8:9.

Shabrani NC, Khan NF, Kale VP, Limaye LS. Polyunsaturated fatty acids confer cryoresistance on megakaryocytes generated from cord blood and also enhance megakaryocyte production from cryopreserved cord blood cells. *Cytotherapy*. 2012;14(3):366–380.

Shannon J, King IB, Moshofskky R, Lampe JW, Gao DL, Ray RM, Thomas DB. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China, *Am J Clin Nutr*; 2007; 85(4): 1090–1097.

Shen Y, Nilsson SK. Bone, microenvironment and hematopoiesis. *Curr Opin Hematol.* 2012; 19(4):250–255.

Siddiqui NF, Shabrani NC, Kale VP, Limaye LS. Enhanced generation of megakaryocytes from umbilical cord blood-derived CD34(+) cells expanded in the presence of two nutraceuticals, docosahexanoic acid and arachidonic acid, as supplements to the cytokine-containing medium. *Cytotherapy*; 2011;13(1):114–128.

Simopoulos AP. Omega-6/omega-3 essential fatty acids: biological effects, *World Rev Nutr Diet*; 2009; 99: 1–16.

Simopouls AP. The importance of the ratio of omega 3/ omega 6 essential fatty acids, *Biomed Pharmacother*; 2002; 56(8): 365–379.

Thangavelu G, Colazo MG, Ambrose DJ, Oba M, Okine EK, Dyck MK. Diets enriched in unsaturated fatty acids enhance early embryonic development in lactating Holstein cows. *Theriogenology* 2007;68:949–957.

Till JE, McCulloch EA. Repair processes in irradiated mouse hematopoietic tissue. *Ann N Y Acad Sci.* 1964;114:115–125.

Turk HF, Chapkin RS. Membrane lipid raft organization is uniquely modified by n-3 polyunsaturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2013;88:43–47.

Uchida N, Weissman IL. Searching for hematopoietic stem cells: evidence that Thy-1.1 lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy1.1 bone marrow. *J. Exp. Med.* 1992; 175:175–184.

Umegaki K, Uramoto H, Suzuki J, Esashi T. Feeding mice palm carotene prevents DNA damage in bone marrow and reduction of peripheral leukocyte counts, and enhances survival following X-ray irradiation. *Carcinogenesis.* 1997;18(10):1943–1947.

Weiss JF, Landauer MR. Radioprotection by antioxidants. *Ann N Y Acad Sci*. 2000;899:44–60.

Xia S, Li XP, Cheng L, Han MT, Zhang MM, Shao QX, Xu HX, Qi L. Fish oil-rich diet promotes hematopoiesis and alters hematopoietic niche. *Endocrinology.* 2015; 156(8):2821-2830.