DRIED PLUM DIET PREVENTS BONE LOSS CAUSED BY IONIZING RADIATION:
REDUCES PRO-RESORPTION CYTOKINE EXPRESSION, AND PROTECTS MARROW-DERIVED OSTEOPROGENITORS

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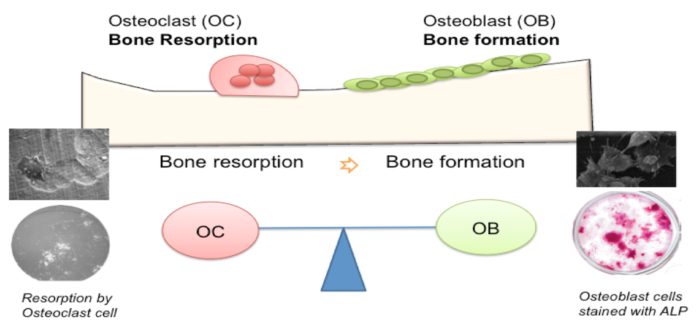
Introduction

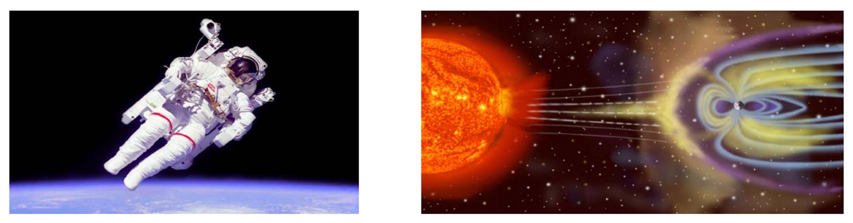
• During prolonged spaceflight, astronauts are exposed to space radiation and are at risk for increased skeletal fragility due to bone loss.
• Evidence shows that ionizing radiation can cause bone loss, due to changes in bone-resorbing osteoclasts (OC) and bone-forming osteoblasts (OB), although the underlying molecular mechanisms for these changes are not fully understood.



• Most bone loss treatments and drugs are anti-resorptive and have undesirable secondary effects, therefore there is still more research to be done in order to find novel anti-osteoporosis treatments.

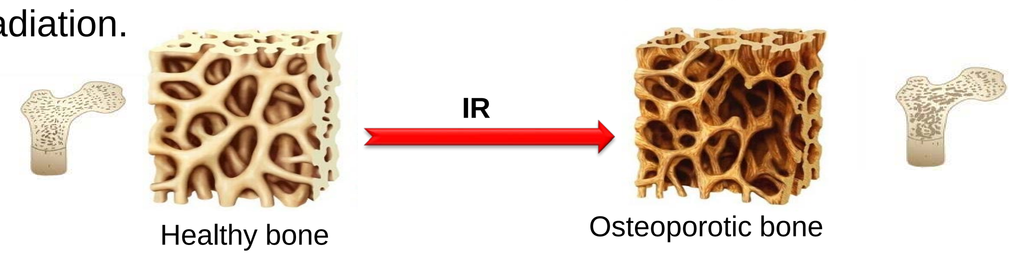
Background

Radiation induces cancellous bone loss
At certain doses, both high and low LET can lead to bone loss.



Gene expression by bone and bone marrow cells:
Measurement of acute and late response and mechanisms
• Pro-osteoclastogenic cytokines: increase expression soon after irradiation (IR) (*Rankl, Mcp1, Tnf*) (*Alwood et al 2015)*
• Oxidative stress: increase in global transcription factor *Nfe2l2* and *Foxo3;* also role of the *mTOR* pathway in oxidative stress
• Cell Cycle and DNA damage: *Cdkn1a* (p21), *p53* and *Gadd45a*

Bone structure and effects of radiation:
Rapid decrease in cancellous bone (spongy bone) followed by radiation.



Dried Plum:
Dried Plum (DP, 25% by weight); mitigates age- and hormone-related bone loss in rodents, although its role as radiomitigant has not been shown until now. Furthermore, the mechanisms are not yet fully understood.

Methods

Mice: Male, C57BL/6J mice,16 wk of age. Mice were separated into groups (n=5-10/group), fed control diet (CD) or Dried Plum Diet (DP) for 14-21 days before irradiation and until sacrifice.

Radiation experiments: Total body irradiation (TBI) of 16 week old mice with 2 Gy Gamma (^{137}Cs @ 83cGy/min, NASA ARC) or 1 Gy Iron (^{56}Fe @5cGy/min, Brookhaven National Laboratory) was performed.

RNA analysis: Total RNA was extracted from bone marrow cells and RNA quality and quantity determined by NanoDrop and Bioanalyzer. Taqman gene expression assays were used. The relative gene expression was quantified using the comparative threshold cycle method with normalization to expression levels of GAPDH or L19.

MicroCT analysis: Tibiae were scanned with 6.7 μm/voxel resolution. To assess bone loss, the bone volume to total volume fraction (BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N,1/mm), and trabecular separation (Tb.Sp, μm) were calculated and reported following conventional guidelines.

Statistics: To assess significant differences, a one-way analysis of variance was performed followed by Dunnett's post-hoc test comparing groups to non-irradiated (sham) controls.

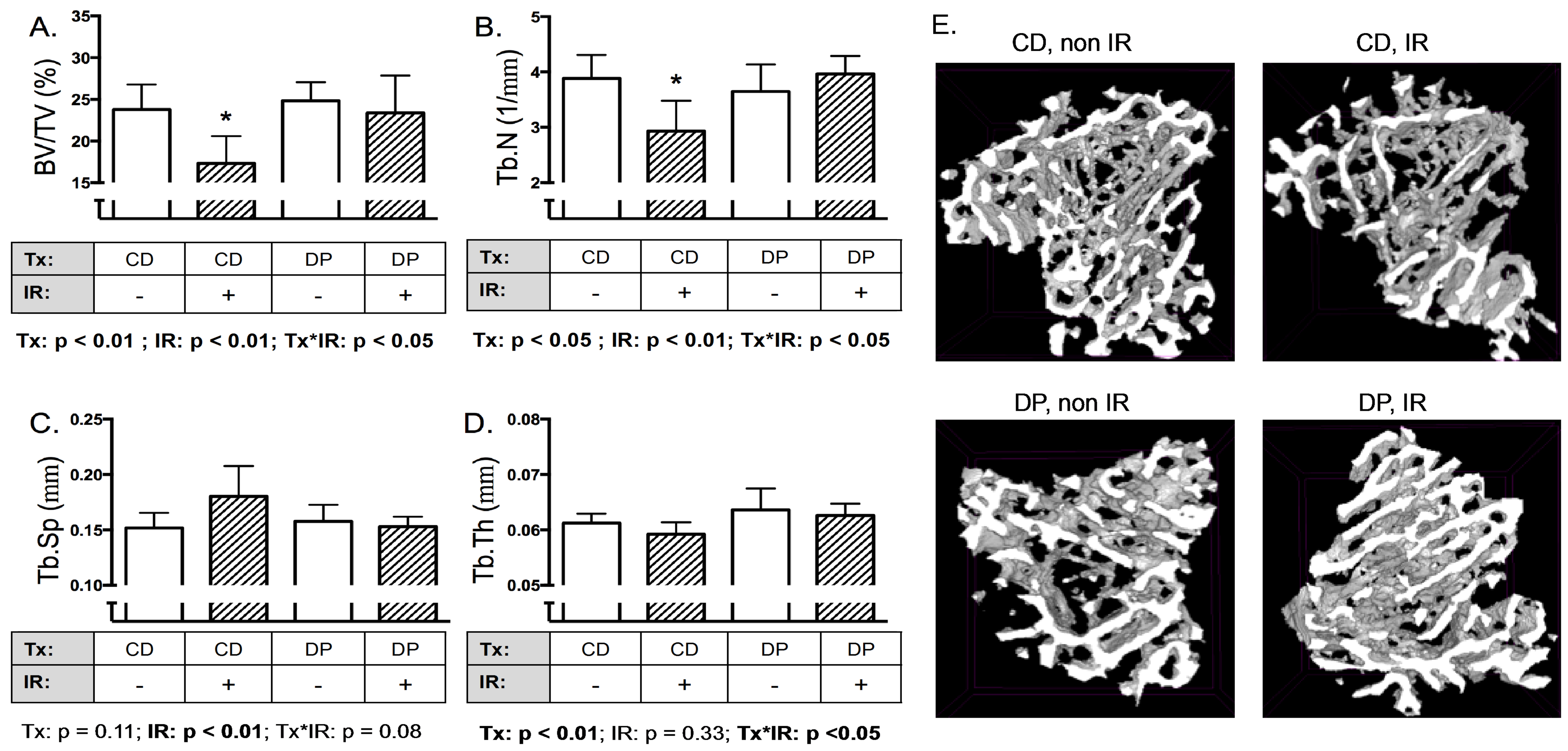


Figure 1: Dried Plum prevents radiation-induced bone loss
Mice were fed control diet (CD) or DP diet, then irradiated with ^{137}Cs (2Gy). After irradiation (11d), mice were euthanized and tibiae collected. Bones were analyzed by microCT for percent bone volume (BV/TV, Panel A), trabecular number (Tb.N, Panel B), trabecular separation (Tb.Sp, Panel C) and trabecular thickness (Tb.Th, Panel D). Representative images of the cancellous bone microarchitecure in 3D reconstructions using the microCT are shown in panel E. Data shown are mean ± S.D. (n=8/group) and analyzed by 2-factor ANOVA. * indicates p<0.05 compared to CD/sham-irradiated controls by Dunnett's post hoc test.
Experiments using high LET (^{56}Fe generated similar results (data not shown).

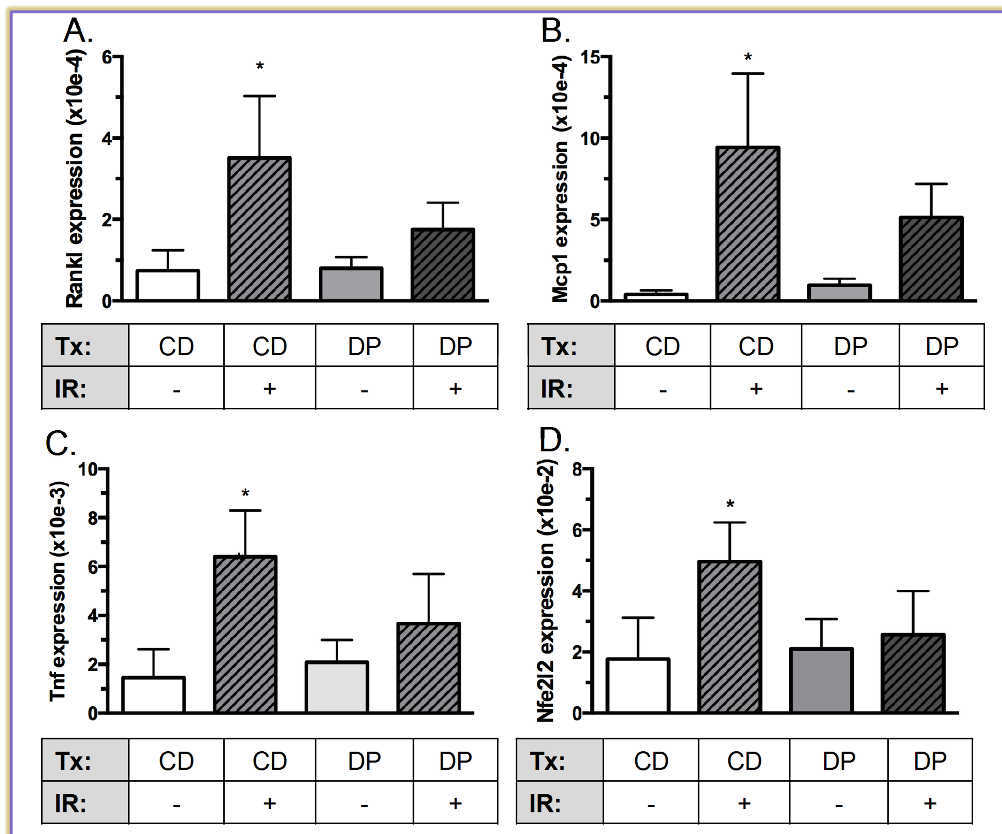


Figure 2. Dried plum reduces radiation-induced changes in expression of resorption-related genes.
Mice were fed CD or DP diets, then irradiated with ^{137}Cs (2Gy). After irradiation (24hr +/- 20min), mice were euthanized and bone marrow cells were collected for analysis of gene expression by qPCR. Y-axis values indicate relative expression levels of gene of interest normalized to *Gapdh* using the ΔCt method. Data shown are mean ± S.D. (n=5-6/group) and analyzed by 1-factor ANOVA. * indicates p<0.05 compared to CD/sham-irradiated controls by Dunnett's post hoc test.

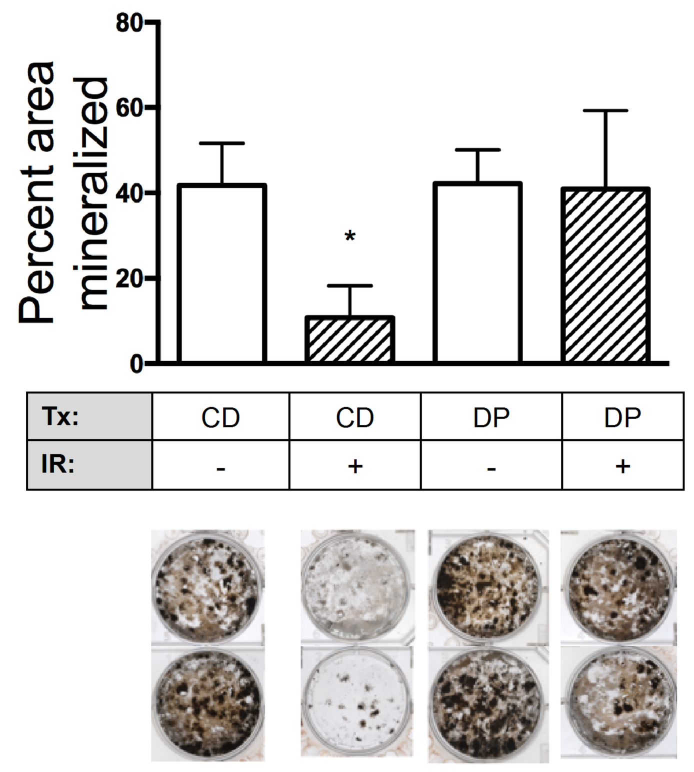
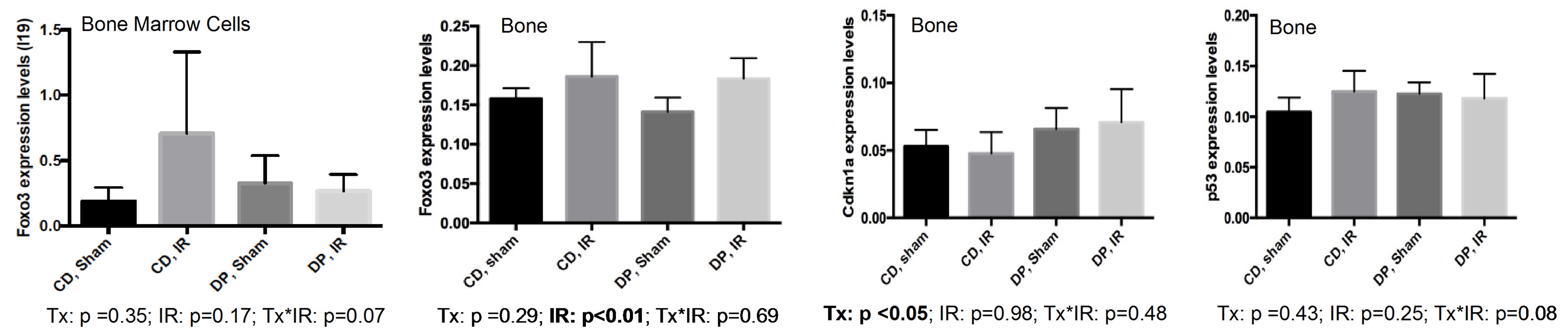


Figure 3: DP protects *ex-vivo* osteoblastogenesis from marrow-derived pre-osteoblasts exposed to 1Gy (^{56}Fe).
After TBI of 1Gy Iron, mice were continuously fed on CD or DP diets until 30 days post IR. Ex-vivo osteoblastogenesis assay was performed, and cells were grown until mineralization (day 21 in culture). Data shown are mean ± S.D. (n=5/group) and analyzed by 1-factor ANOVA. * indicates p<0.05 compared to CD/sham-irradiated controls by Dunnett's post hoc test.



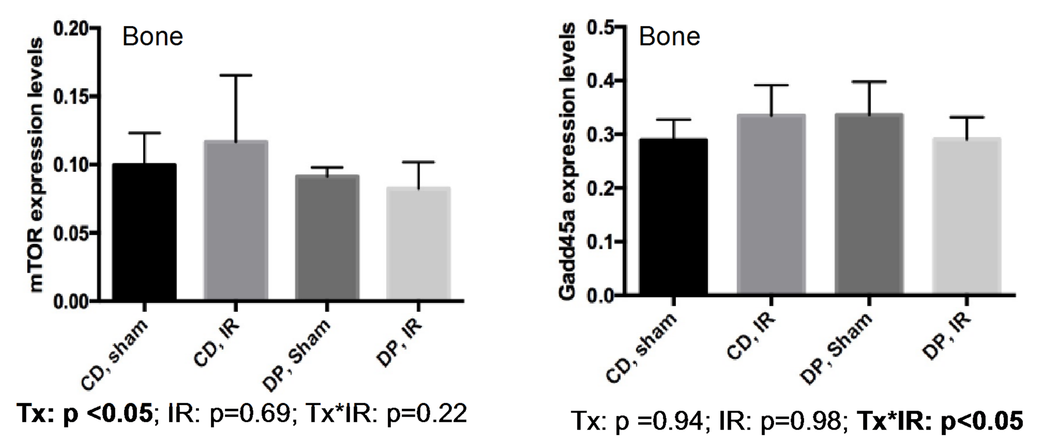


Figure 4. Potential role of Dried Plum in modulating oxidative stress response. Mice were fed various the CD or DP diets (treatment, Tx), then irradiated with ^{137}Cs (2Gy, IR). 11 days after irradiation, mice were euthanized, and bone and bone marrow cells were collected for analysis of gene expression by qPCR. Y-axis values indicate relative expression levels of gene of interest normalized to *L19* using the ΔCt method. Data shown are mean ± S.D. (n=6-8/group) and analyzed by 2-factor ANOVA. * indicates p<0.05 compared to CD/sham-irradiated controls by Dunnett's post hoc test.

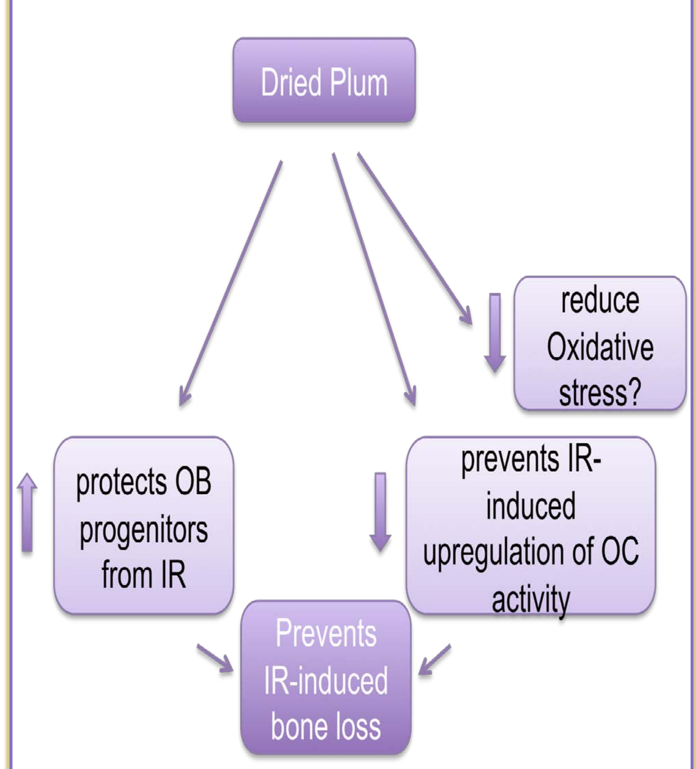


Figure 5. Hypothetical model on how DP protects bone from radiation.

Summary and Conclusions

• Dried Plum (DP) prevents both low-LET and High-LET radiation-induced cancellous bone loss.
• DP diet reduces the early increase in pro-resorption cytokines, blunting the early stages of bone loss.
• DP protects osteoblasts progenitor, as observed by enhanced ex-vivo osteoblast mineralization capacity. Data suggests that DP has a beneficial effect on osteoblasts.
• DP appears to modulate oxidative stress and cell cycle responses at early (1 day post IR, *Nf2l2*) and later stages (11 days post IR, *Gadd45a*, *mTOR*, *Cdkn1a*).
• DP's mode of action may be exerted via its antioxidant, polyphenolic and iron-chelating components.

• **Conclusion: Dried plum is a promising candidate radiomitigant for bone.**

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