

<b>Fiscal Year:</b>	FY 2023	<b>Task Last Updated:</b>	FY 02/24/2023
<b>PI Name:</b>	Rithidech, Kanokporn Ph.D.		
<b>Project Title:</b>	Countermeasures Against Adverse Effects of Space Radiation		
<b>Division Name:</b>	Human Research		
<b>Program/Discipline:</b>			
<b>Program/Discipline-- Element/Subdiscipline:</b>			
<b>Joint Agency Name:</b>		<b>TechPort:</b>	No
<b>Human Research Program Elements:</b>	(1) <b>SR</b> :Space Radiation		
<b>Human Research Program Risks:</b>	(1) <b>Cancer</b> :Risk of Radiation Carcinogenesis		
<b>Space Biology Element:</b>	None		
<b>Space Biology Cross-Element Discipline:</b>	None		
<b>Space Biology Special Category:</b>	None		
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<b>Comments:</b>			
<b>Project Type:</b>	GROUND	<b>Solicitation / Funding Source:</b>	2017-2018 HERO 80JSC017N0001-BPBA Topics in Biological, Physiological, and Behavioral Adaptations to Spaceflight. Appendix C
<b>Start Date:</b>	01/31/2019	<b>End Date:</b>	09/30/2023
<b>No. of Post Docs:</b>	1	<b>No. of PhD Degrees:</b>	
<b>No. of PhD Candidates:</b>		<b>No. of Master' Degrees:</b>	
<b>No. of Master's Candidates:</b>		<b>No. of Bachelor's Degrees:</b>	
<b>No. of Bachelor's Candidates:</b>		<b>Monitoring Center:</b>	NASA JSC
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<b>Flight Program:</b>			
<b>Flight Assignment:</b>	NOTE: End date changed to 9/30/2023 per NSSC information (Ed., 5/18/23) NOTE: End date changed to 5/30/2023 per NSSC information (Ed., 5/8/22) NOTE: End date changed to 5/30/2022 per NSSC information (Ed., 3/29/21)		
<b>Key Personnel Changes/Previous PI:</b>			
<b>COI Name (Institution):</b>			
<b>Grant/Contract No.:</b>	80NSSC19K0435		
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<b>Performance Goal Text:</b>			

**Task Description:**

This is the task book for the Phase 1 study of our project entitled “Countermeasures against adverse effects of space radiation” in which only male mice are included. The search for efficient radiation countermeasures in space is a high priority. Apigenin (AP), a natural flavone, displays potent antioxidant, anti-inflammatory, anti-microbial, and anti-neoplastic activities. We found that AP protects human lymphocytes from in vitro  $\gamma$ -irradiation and that intraperitoneal AP diminishes inflammation in the bone marrow (BM) of  $\gamma$ -irradiated mice. However, the countermeasure activity of AP has not been studied for space radiation. Here we report the efficacy of oral AP for space-radiation-induced toxicity in hematopoietic and gut tissues. We gave food with (20 mg/kg/bw) or without (0 mg/kg/bw) AP to male C57BL/6 mice (with or without irradiation) before (5 days) and after total-body exposure to 0 or 0.5 Gy of 28Si ions. There were 4 experimental groups. Mice with the AP diet (with or without irradiation) were maintained on the AP diet until mice reach 620 days of age (at which time the weights of mice in each group did not significantly change) before switching to a regular diet. At 1-week after-irradiation, 6 mice from each group were randomly euthanized to test AP countermeasure for space-radiation-induced early toxicity (hematological toxicity and inflammation) in BM cells and the duodenum (the main absorption site for oral AP) collected from the same mouse. We also evaluated the effectiveness of AP in protecting/mitigating mice from Si-induced chromosome aberrations (using the mouse blood in vivo micronucleus assay). Our data enable the evaluation of the countermeasure efficacy of AP across tissues at risk for cancer induction, i.e. bone marrow, the lung, and the gastrointestinal (GI) tract. Importantly, we used the 16S rRNA amplicon sequencing method to study the impact of the AP diet on the gut microbiota of mice with or without exposure to silicon ions. This multi-tissue of the same exposed individual approach has not been used in space research. All remaining mice were observed for morbidity and mortality until they reach about 770 days of age to determine the effectiveness of AP in counteracting Si-induced cancer.

**Rationale for HRP Directed Research:****Research Impact/Earth Benefits:**

People can be exposed to radiation in many different ways, both intentionally or unintentionally, on earth or in the space environment. Hence, the search for efficient medical countermeasures (protectors, those given before exposure; mitigators, those given after exposure) is a high priority in radiation protection on earth and space. The ideal biological countermeasure for counteracting harmful effects of radiation should be (i) capable of suppressing inflammation (one of the important physiological changes related to untoward health outcome) after radiation exposure, (ii) enabling the prevention and mitigation of radiation-induced damage across tissues, (iii) amenable to apply before the exposure, regularly, and after radiation exposure, and (iv) easily administered (i.e. oral route which is compatible with self-administration and dosing in space and the battlefield). However, despite significant efforts, and advances in developing radiation countermeasures, both protectors and mitigators are still an unmet need. It is therefore important to continue the search for efficient countermeasures to protect the general population in the event of nuclear terrorism or accident, as well as on the battlefield (in the event of radiological explosive devices being used), as well as astronauts and space travelers. Hence, our findings of the effective countermeasure activity of AP given as a diet supplement for Si-induced toxicity to the hematopoietic and the GI would have a significant impact on radiation protection during the space mission and on earth. Our data on the impact of the AP diet on the gut microbiota of mice with or without exposure to silicon ions would significantly have an important impact in optimizing the diet with AP, as well as integration with other bioactive compounds (e.g. other flavonoids and polyphenols), pre-biotic and pro-biotic compounds for potentially synergistic beneficial effects to protect the general population or patients who will be undergoing or have undergone radiation therapy. Further, our data on the efficacy of AP in the protection/prevention and/or mitigation of radiation-induced cancer will have a significant impact on the future development of a novel therapeutic strategy to prevent or mitigate the acute or long-term effects of radiation during a space mission, in the event of radiobiological events on earth, (e.g. a nuclear accident or terrorisms) or in the battlefield.

**Task Progress:**

We terminated the Phase 1 study when all remaining mice reached about 770 days of age. At necropsy, a gross examination was performed to identify potential lesions in several organs. Several tissues (i.e., sternum, spleen, gut, kidney, liver, lung, and thymus) were collected from mice with lesions and fixed in 10% formalin for further histopathological evaluation.

We have completed the analyses of the countermeasure effectiveness of apigenin (AP) in several tissues collected from the same male C57BL/6 mice at 7 days after exposure to 0 (sham controls) or 0.5 Gy of 260 MeV/n 28Si ions (delivered at 0.5 Gy/min) with or without the AP-diet. These tissues included blood, bone marrow cells, and gut tissues. Our results show that AP can:

- prevent the loss of white blood cells (leukopenia),
- prevent the depletion of platelets (thrombocytopenia),
- increase the production of red blood cells (erythropoiesis),
- enhance the proliferation of hematopoietic stem/progenitor cells,
- reduce Si-induced chromosome aberrations (assayed by the reduction in the frequencies of blood micronuclei, and
- suppress inflammation in the bone marrow (BM) and the gut tissues, assayed by the levels of activated-Nuclear factor- $\kappa$ B (NF- $\kappa$ B) and the levels of expression of pro-inflammatory cytokines

These sets of data have been published in Life Sciences in Space Research. Furthermore, the data also were presented at the NASA Human Research Program Investigators' Workshop (HRP IWS) conferences and the Research Day of the Department of Pathology, SBU, Stony Brook, NY. We continue to investigate the impact of the AP diet on the gut microbiota and cancer incidence in mice with or without exposure to silicon ions.

In addition to the hematopoietic tissues, our results indicate that the AP diet protects mice from Si-induced injuries in the gastrointestinal (GI) tract. Hence, our data demonstrate the countermeasure potential of dietary AP across tissue (hematopoietic and gut tissues) due to anti-inflammatory activity. This multi-tissue of the same exposed individual approach has not been used in space research. Overall, In summary, based on our data using male mice as an animal model, AP is a promising countermeasure for space radiation-induced tissue injuries and warrants further investigation of the countermeasure effectiveness of AP for radiation-induced injuries in female mice (Phase 2 study). This is important since gender differences in response to radiation exposure have been demonstrated. Further, differences in drug metabolisms between males and females have also been found.

**Bibliography Type:**

Description: (Last Updated: 05/17/2023)

Abstracts for Journals and Proceedings	<p>Peanlikhit T, Honikel L, Liu J, Li L, Zimmerman T, Rithidech K. "Effects of apigenin on the gut microbiome of mice exposed to silicon ions." Annual meeting of NASA Human Research Program Investigators' Workshop (virtual), February 7-10, 2022.</p> <p>Abstracts. 2022 NASA Human Research Program Investigators' Workshop, Galveston, February 7-10, 2022. , Feb-2022</p>
Abstracts for Journals and Proceedings	<p>Peanlikhit K, Li J, Liu J, Honikel L, Zimmerman T, Rithidech K. "Chemoprevention of apigenin for space radiation-induced acute leukemia and lymphoma in mice." Annual Retreat/Marvin Kuschner, Pathology Department, November 2022.</p> <p>Abstract. Annual Retreat/Marvin Kuschner, Pathology Department, November 2022. , Nov-2022</p>
Abstracts for Journals and Proceedings	<p>Rithidech, K, Peanlikhit T, Honikel L, Liu J, Li J, Zimmerman T, Welsh J. "Apigenin: A promising countermeasure for space-radiation-induced damage." Radiation Research Society's 68th Annual Meeting, Hawaii, October 15-19, 2022.</p> <p>Abstract. Radiation Research Society's 68th Annual Meeting, Hawaii, October 15-19, 2022. , Oct-2022</p>
Articles in Peer-reviewed Journals	<p>Peanlikhit T, Honikel L, Liu J, Zimmerman T, Rithidech K. "Countermeasure efficacy of apigenin for silicon-ion-induced early damage in blood and bone marrow of exposed C57BL/6J mice." Life Sciences in Space Research. Life Sci Space Res (Amst):35:44-52. <a href="https://doi.org/10.1016/j.lssr.2022.05.007">https://doi.org/10.1016/j.lssr.2022.05.007</a> ; PubMed <a href="#">PMID: 36336369</a> , Nov-2022</p>