Dear Editor in Chief of Pharmaceutical Research,

We would like to submit our original manuscript titled “Microbiome and metabolome alterations in Nrf2 knockout mice with induced gut inflammation and fed with phenethyl isothiocyanate and cranberry enriched diets” to the Pharmaceutical Research journal. This is an original manuscript and has not been published previously in any language anywhere, nor it is under simultaneous consideration by any other journal. This work was carried out by Ran Yin, Davit Sargsyan, Renyi Wu, Rasika Hudlikar, Shanyi Li, Hsiao-Chen Kuo, Md Shahid Sarwar, Yuyin Zhou, Zhan Gao, Amy Howell, Chi Chen, Martin J. Blaser and Ah-Ng Kong.

We believe that our manuscript is suitable for publication in *Pharmaceutical Research* as it is exploring compositional changes in gut microbiota under multiple conditions including irritable bowel disease animal model and potentially anti-inflammatory diets. IBD was modeled in mice via dextran sulfate sodium (DSS) administration, creating inflammation in the gastrointestinal (GI) tract. We tested cranberry and phenethyl isothiocyanate (PEITC) enriched diets against the standard grain diet in C57BL/6J wild type (WT) and Nrf2 gene knock-out (KO) mice. Nrf2 pathway is a master regulator of oxidative stress and inflammation. Cranberries are rich in antioxidants and can help prevent bacterial infections, while PEITC is found in cruciferous vegetables and has anti-cancer and anti-inflammatory properties. Incorporating these into diet may have potential health benefits for human gut. Microbes and metabolites interactions play crucial roles in maintaining GI tract balance.

We showed that Nrf2 KO mice had higher alpha diversity compared to WT. Cranberry and PEITC limited the effect of DSS and increased the diversity of mice gut microbiota. DSS challenge altered the production of several metabolites while PEITC and cranberry feeding prevented these changes. The enriched diets modulated the metabolic responses to induced inflammation likely via microbial composition alterations. Nrf2 KO mice had lower levels of short-chain fatty acids (SCFA) and amino acids such as glutamate, phenylalanine and prolin, and higher levels of secondary bile acids such as DCA, LCA and MCA compared to WT mice. Additionally, the dietary supplements showed the reversal of the negative effect of DSS-induced inflammation on the balance of Firmicutes and Bacteroidetes phylum in the hosts’ intestines.

Thank you for your consideration.

Sincerely,

Davit Sargsyan