

Healthy Gut! Healthy You!: Preclinical Validation of a Novel Enzyme Therapy for Improving Generalized Anxiety Disorder and Overall Gastrointestinal Health in *Rattus norvegicus*

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As of 2022, 49% of U.S. adolescents and 25% of adults have psychological disorders, with 60% also experiencing gastrointestinal disorders. The aim of the current study is to determine whether the enzymes Papain, Bromelain, and Amylase can improve the negative impacts of Generalized Anxiety Disorder (GAD) while helping improve overall gastrointestinal health. The study also explored IL-4 as potential biomarker for anxiety. The rat model was employed to explore the most translational model. Rats who were 30 days postnatal were shipped from Indianapolis to Colorado Springs, a condition that naturally creates anxious behaviors. They were randomly assigned to the Control group (C), Papain/Bromelain/Amylase (PBA) group, or the Papain/Bromelain (PB) group. After being given the enzymes through drinking water for one week, the rats' anxious behavior was tested using the Elevated Plus Maze (EPM) test. This test relies on exploration and does not cause additional stress to the rodents. Additionally, rat fecal samples were taken for 16SrRNA sequencing to analyze species diversity and understand differences in the composition of the gut microbiota. Behavioral results indicate that the PBA group consistently displayed decreased anxious behaviors compared to the control and PB groups. In 16SrRNA sequencing, the PBA group was significantly different compared to the others in overall gut microbiota composition. However, there were no differences in dominant species diversity between groups. When the ELISA was conducted, there was no difference in IL-4 levels presented between test groups. Future studies should employ more rigorous models of testing to more clearly understand microbial alteration and test for other potential biomarkers such as IL-2 or IL-8.

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