Indonesian Journal of Pharmacy

VOL 37 (3) 2026: 555–565 | RESEARCH ARTICLE

Potential Preventive Effects of *Ficus carica* Puree from Reduced Number of Ileal Microbiota and Tissue Damage on Ileal and Hippocampal Acute Hypoxia-induced Sprague–Dawley Rats

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Article Info

Submitted: 02-01-2025
Revised: 03-07-2025
Accepted: 08-07-2025

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ABSTRACT

Acute hypoxia (AH) induces excessive reactive oxygen species production, contributing to intestinal epithelial barrier dysfunction and neurological injury. This study evaluated the potential preventive effects of whole *Ficus carica* administered as a puree (FCP) in promoting gut microbiota growth and modulating gut–brain axis function. Twenty-four male Sprague–Dawley rats were randomly assigned into the following six groups: normoxia control, AH + corn oil (AHC, 1 mL/200 g body weight/day), AH + vitamin E (1 mL/200 g body weight/day), and three AH + FCP treatment groups (AHF1 [1 mL/200 g], AHF2 [2 mL/200 g], and AHF3 [4 mL/200 g]). Treatments were orally administered for 28 days before AH induction, which was conducted via exposure to a hypoxic chamber (90% N_2 and 10% O_2) for 4 hours. On the final day, cecal contents and ileal and brain tissues were collected for histopathological assessment and bacterial quantification, including total plate counts of lactic acid bacteria (LAB), *Escherichia coli* (*E. coli*), and coliforms. Hematoxylin–eosin staining revealed reduced inflammatory cell infiltration and the absence of histological damage in the ileum and hippocampus of the AHF1, AHF2, and AHF3 groups, in contrast to the AHC group. The AHF1 and AHF3 groups exhibited significantly higher LAB and coliform populations than the AHC group, whereas *E. coli* counts were notably elevated in AHF1. These results suggest that FCP exerts protective effects against AH-induced intestinal and neural damage by preserving microbiota composition and supporting gut–brain axis regulation.

Keywords: acute hypoxia, *Ficus carica* puree, intestinal microbiota, hippocampus, gut–brain axis

INTRODUCTION

Normoxia, or normal oxygen availability, is crucial for the survival of aerobic mammals, as oxygen is essential for mitochondrial respiration. Within the mitochondria, oxygen drives cellular metabolism by facilitating oxidative phosphorylation, where adenosine diphosphate is converted into adenosine triphosphate, generating carbon dioxide and water as byproducts (Lee et al., 2020). In contrast, hypoxia denotes a state of reduced oxygen tension, frequently ranging from 6 to 34 mmHg (1%–5% O_2), impairing cellular oxygen availability and disrupting physiological processes (Lee et al., 2020; Sarkar et al., 2017). Cell type, metabolic demand, and intrinsic adaptive capacity influence the extent of hypoxia-induced cellular damage (Chen et al., 2020; Macintyre, 2014). At the cellular level, hypoxia disrupts metabolic pathways through various mechanisms, including impaired oxidative phosphorylation, enzyme dysfunction, and energy depletion. These events promote excessive reactive oxygen species

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(ROS) production, triggering oxidative stress, cellular injury, and inflammation, which may ultimately result in organ dysfunction (Chu et al., 2023; David et al., 2021; Lee et al., 2020; Villa et al., 2023). Oxygen availability is significantly involved in gastrointestinal homeostasis regulation, particularly intestinal absorption and epithelial barrier maintenance. Under hypoxic conditions, increased ROS levels contribute to intestinal barrier dysfunction and worsen inflammatory responses. This disruption has been associated with the pathogenesis of inflammatory bowel disease and other gastrointestinal disorders, as well as perturbations in the gut microbiota composition (Aviello & Knaus, 2017; Fitri et al., 2024; Luo et al., 2023; Meliala et al., 2024; Widyawati et al., 2025). Moreover, hypoxia-induced inflammation can extend beyond the gut to the central nervous system (CNS), causing cerebral edema, blood–brain barrier disruption, and neuronal damage (Zhou et al., 2017). Inflamed tissue represents a hallmark of hypoxia caused by vascular dysfunction and immune cell infiltration, collectively contributing to a cycle of hypoxia and sustained inflammation (Dvornikova et al., 2023).

Gastrointestinal hypoxia is a major contributor to intestinal inflammation, frequently arising from aberrant immune responses to luminal antigens and microbial population dysregulation (Dvornikova et al., 2023). During digestion, a dynamic interaction between intestinal epithelial cells and resident microbiota is critical for absorbing nutrient and regulating oxygen gradients along the gut mucosa (Konjar et al., 2021). These interactions play a significant role in maintaining intestinal homeostasis, including barrier integrity, immune modulation, and metabolic processing. In healthy adults, the gut microbiota predominantly comprises obligate anaerobes, creating a low-oxygen environment optimal for their growth (Lee et al., 2020). However, shifts in oxygen levels and microbial composition, particularly the overgrowth of facultative aerobes, can disrupt epithelial function and initiate proinflammatory cascades, exacerbating gastrointestinal pathology (Dvornikova et al., 2023). The human intestinal microbiome contains several bacterial phyla, including Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, and Spirochaetes (Markowiak-Kope & Slizewska, 2020). Among these phyla, gram-positive Firmicutes (e.g., *Lactobacillus* spp., *Bacillus* spp., *Clostridium* spp.) and gram-negative Bacteroidetes represent the dominant constituents of the adult gut microbiota (Joseph et al., 2019; Sun et al., 2018; Zheng et al., 2021). These microbes possess specialized metabolic capacities that facilitate the degradation of complex carbohydrates, including cellulose, hemicellulose, pectin, mucin, and lignin, into simpler sugars. Subsequently, these are fermented into short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which function as key modulators of the gut and brain physiology (Dalile et al., 2019; Markowiak-Kope & Slizewska, 2020).

Dietary intake of fermentable fibers, prebiotics, and probiotics, which promote the growth of beneficial SCFA-producing bacteria, enhance SCFA production. These metabolites influence the gut–brain axis through several pathways, including immunological, endocrine, vagal, and humoral, thereby exerting systemic effects on inflammation, oxidative stress, and neural signaling (Dalile et al., 2019). Accordingly, preserving the composition and function of the gut microbiota is increasingly recognized as a critical factor in gastrointestinal and neurological health maintenance. Functional foods, including *Ficus carica* (fig), are rich sources of dietary fiber and antioxidants, including phenolic and flavonoid compounds. Figs puree (FCP) has been reported to contain total phenolic and flavonoid contents of 0.0143%–0.0150% and 0.2190%–0.2201%, respectively, along with a high dietary fiber content of approximately 9.32 g/100 g (Meliala et al., 2024). Previous studies have indicated that dietary intake of FCP can enhance SCFA production by modulating intestinal microbiota activity (Markowiak-Kope & Slizewska, 2020; Meliala et al., 2021). Moreover, the antioxidant constituents in figs have been demonstrated to increase plasma lipoprotein levels and mitigate oxidative damage by neutralizing free radicals. Therefore, figs' extracts or purees may act as potent oxidative stress inhibitors owing to their high antioxidant capacity (Meliala et al., 2024). Despite these promising bioactivities, studies investigating the application of figs in mitigating the pathophysiological consequences of acute hypoxia (AH) are lacking. In our previous study, we noted that figs reduced AH-induced damage through mechanisms involving the modulation of antioxidant enzyme activity and secondary metabolite pathways. Building upon these findings, the present study aimed to evaluate the potential preventive effects of FCP on intestinal microbiota

composition and its role in modulating the gut–brain axis function under AH conditions.

MATERIALS AND METHODS

This is a quasi-experimental study that aimed to evaluate the preventive effects of FCP using a posttest-only controlled group design. This study was conducted at the Experimental Animal Laboratory, Department of Pharmacology, Faculty of Medicine, Public Health and Nursing, and the Animal Physiology Laboratory, Faculty of Biology, Gadjah Mada University (Figure 1).

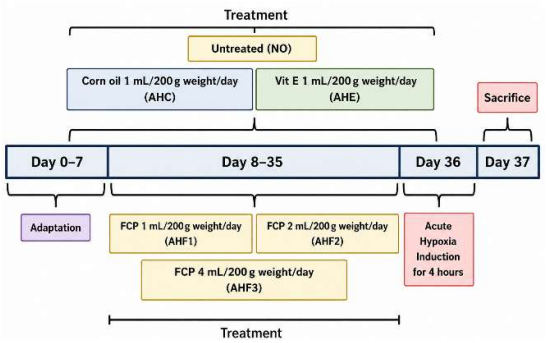


Figure 1. Experimental Design.

FCP Preparation

Fresh fig was obtained from CV. Jordan, Bandung, West Java, Indonesia. The following procedures were used for preparing FCP: washing, slicing, crushing, and homogenizing for 20 min at a speed of 8,000 rpm using an Armfield L4R homogenizer. The samples were placed in an airtight container and stored in a refrigerator at 2°C without light for a maximum duration of 3 days. FCP preparation was repeated at 3-day intervals (Meliala et al., 2024).

Acute Hypoxia Induction in an Animal Model

Twenty-four Sprague–Dawley rats (aged 2–3 months and weighing 180–200 g) were obtained from the Faculty of Medicine and Public Health, Muhammadiyah University, Yogyakarta, Indonesia. They were divided into the following six groups (n = 4): NO: normoxia control (no treatment, normal oxygen exposure); AHC: AH + corn oil (Tropicana Slim®; 1 mL/200 g body weight/day); AHE: AH + vitamin E (EMPROVE®; 1 mL/200 g body weight/day); AHF1: AH + FCP (1 mL/200 g body weight/day); AHF2: AH + FCP (2 mL/200 g body weight/day); AHF3: AH + FCP (4 mL/200 g body weight/day).

FCP, corn oil, or vitamin E was orally administered for 28 consecutive days (days 8–35). On day 36, AH was induced by placing animals in a custom-built hypoxia chamber (25 cm × 15 cm × 15 cm) filled with a gas mixture containing 90% nitrogen (N_2) and 10% oxygen (O_2) for 4 hours. On day 37, the animals were euthanized, and ileal and brain tissues were collected for histopathological and cecal contents for microbiological analyses (Meliala et al., 2024).

Hematoxylin–Eosin Staining

Histological analysis of ileal and hippocampal tissues was performed using hematoxylin–eosin (HE) staining, following the method of Cai et al. with minor modifications (Cai et al., 2018). Tissue samples were sectioned at a 3-µm thickness and underwent deparaffinization by immersion in three sequential xylene baths (Xylol I, II, and III) for 3 min each. Rehydration was subsequently conducted by passing the sections through a graded ethanol series (100%, 95%, 80%, and 70%) for 2 min per concentration, followed by rinsing under running tap water for 3 min. This process is necessary because paraffin-embedded tissue cannot directly absorb the dye because paraffin is hydrophobic, thus blocking the penetration of water-based dyes, including hematoxylin and eosin. By removing the paraffin using solvents, such as xylene, the tissue opens and can undergo a rehydration process to facilitate optimal penetration of the dye into the tissue.

Nuclear staining was achieved by immersing the sections in Mayer's hematoxylin solution for 7 min, followed by an additional 7-min rinse in running water. Cytoplasmic counterstaining was performed by dipping the sections in eosin solution for approximately 30 s at room temperature. The stained sections were briefly rinsed by dipping in three sequential baths of distilled water to remove excess stain. Dehydration was repeated using ascending alcohol concentrations (70%, 80%, 95%, and 100%), with three dips per concentration. Subsequently, the slides were cleared in two changes of xylene (2 min each). Finally, the sections were mounted using one drop of Entellan and covered with a glass coverslip for microscopic evaluation.

Total Plate Count

To assess the microbial composition of the cecum, serial dilutions ranging from 10^{-1} to 10^{-8} were prepared by aseptically transferring 1 mL of homogenized cecal content into 9 mL of sterile

0.9% NaCl solution, followed by vortexing. For lactic acid bacteria (LAB) enumeration, 1 mL of each dilution was plated onto sterile petri dishes using the pour plate method, with de Man–Rogosa–Sharpe (MRS) agar (Difco®, Detroit, MI, USA) added while still in a liquid state (Mantegazza et al., 2023). Plates were incubated at 30°C for 48 h.

To determine the total plate counts (TPCs) of *Escherichia coli* (*E. coli*) and coliforms, chromogenic agar (Oxoid®) and MacConkey agar (Becton Dickinson®), respectively, were used. The agar media were poured aseptically onto petri dishes containing 1 mL of diluted sample using the same pour plate technique and incubated at 37°C for 24 h (Filius et al., 2003). Colony growth was enumerated and expressed as colony-forming units per milliliter, using an automated colony counter (Biobase®).

Statistical Analysis

Microbial count data were initially tested for normality and homogeneity using the Shapiro–Wilk and Kruskal–Wallis tests, respectively. Owing to the nonnormal distribution and heterogeneity of the data, nonparametric Mann–Whitney U tests were employed for pairwise comparisons among groups. Results were presented as means ± standard deviations. $P < 0.05$ was considered statistically significant, and means labeled with different superscript letters within the same column indicate statistically significant differences. All statistical analyses were performed using SPSS version 27 (IBM Corp., Chicago, IL, USA). Histopathological data from intestinal and brain tissues were descriptively analyzed on the basis of the degree and nature of tissue damage.

RESULTS AND DISCUSSION

HE staining was performed for detecting acidosis of inflammatory response and abnormal morphology in ileal (Figure 2) and hippocampal tissues (Figure 3) following AH. In the NO group, HE staining of the ileal tissue did not reveal inflammatory cells in the lamina propria (Figure 2A). However, in the AHC and AHE groups (Figures 2B and 2C, respectively), remarkable inflammatory cell infiltration was observed from the lamina propria to the intestinal lumen. Some inflammatory cells infiltrated the epithelial crypts, whereas some cells underwent degeneration. Meanwhile, in the AHF1 (Figure 2D), AHF2 (Figure 2E), and AHF3 groups (Figure 2F), slight inflammatory cell infiltration was noted in the lamina propria (Figure 2A). However, in the AHC and AHE groups (Figures 2B and 2C, respectively), marked inflammatory cell infiltration was detected from the lamina propria to the intestinal lumen. Some inflammatory cells infiltrated the epithelial crypts, whereas some cells underwent degeneration. Meanwhile, in the AHF1 (Figure 2D), AHF2 (Figure 2E), and AHF3 groups (Figure 2F), slight inflammatory cell infiltration was observed in the lamina propria.

In the NO group, hippocampal neural cell architecture appeared intact without evidence of necrosis (Figure 3A). In contrast, the AHC group demonstrated preserved neural cell morphology accompanied by substantial lymphocyte infiltration, without necrotic changes (Figure 3B). Similarly, the AHE group maintained normal neural structure and exhibited only mild lymphocyte infiltration, with no necrosis observed (Figure 3C). Moreover, the AHF1, AHF2, and AHF3 groups demonstrated normal neural cell morphology with mild lymphocytic infiltration and absence of necrosis (Figures 3D–F). The TPC of LAB in the NO group was comparable to those in the AHF1 and AHF3 groups and was significantly higher than those in the AHC, AHE, and AHF2 groups (Figure 4). LAB metabolizes sugars to generate lactic acid, which dissolves calcium carbonate in the culture medium, resulting in a clear zone surrounding colonies (Mohamad et al., 2020; Nur et al., 2017). Colonies of LAB on MRS agar are frequently round, convex, cream-colored, and lack prominent pigmentation (Taye et al., 2021).

Conversely, the TPC of *E. coli* in the NO group was similar to that in the AHF3 group and significantly greater than that in the AHC, AHE, AHF1, and AHF2 groups. *E. coli* produces β-glucuronidase, an enzyme that hydrolyzes chromogenic substrates in selective agar media, causing colonies to appear purple (Farfour, 2019; Meddeb et al., 2014). Coliform bacterial counts followed a pattern akin to that of LAB (Figures 4 and 5). On MacConkey agar, coliform colonies demonstrate a characteristic pink coloration owing to lactose fermentation. This medium selectively supports gram-negative bacteria by incorporating bile salts and crystal violet, inhibiting gram-positive bacterial growth, facilitating prompt coliform identification (Devaraj et al., 2016; Supriatin et al., 2021).

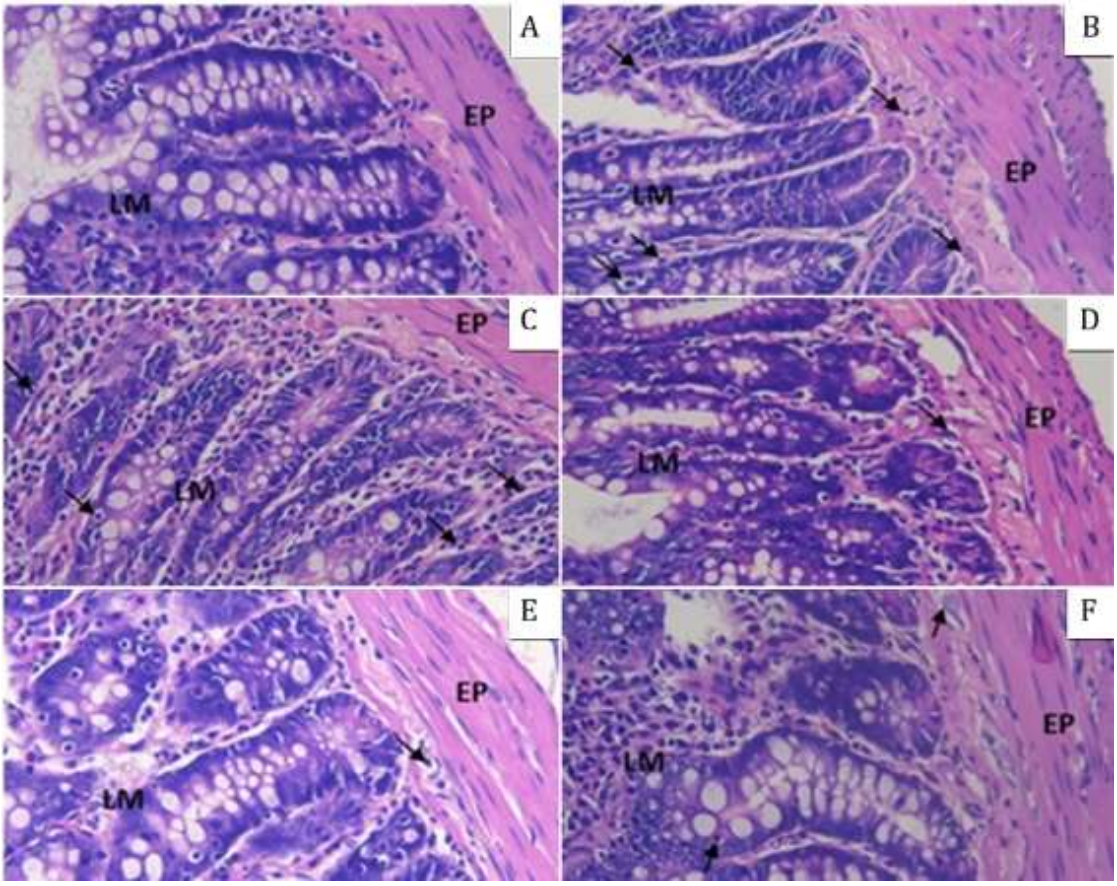


Figure 2**.** Representative hematoxylin–eosin (HE) staining of rat ileal tissue. Inflammatory cells are indicated by arrows (→). Panels: (A) Normoxia control (NO); (B) Acute hypoxia+ corn oil 1 mL/200 g body weight/day (AHC); (C) Acute hypoxia + vitamin E 1 mL/200 g body weight/day (AHE); (D) Acute hypoxia + *Ficus carica* puree (FCP) 1 mL/200 g body weight/day (AHF1); (E) Acute hypoxia + FCP 2 mL/200 g body weight/day (AHF2); (F) Acute hypoxia + FCP 4 mL/200 g body weight/day (AHF3). LM: lamina propria; EP: epithelial cells. All images at 400× magnification.

AH represents an environmental-related stress characterized by reduced oxygen availability (1%–5% O_2), causing excessive oxidative stress, elevated ROS production, and metabolic disruption at tissue and organ levels (Duvillié & Jockers, 2023; Lee et al., 2020). Hypoxia induces the stabilization of hypoxia-inducible factors, which bind to hypoxia response elements, eliciting the transcription of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α (Zhang et al., 2006). The consequent increased systemic inflammatory mediator levels contribute to dysfunction in several organs, particularly the intestine and brain (Eltzschig & Carmeliet, 2011; Mukandala et al., 2016).The steep oxygen gradient between the anaerobic intestinal lumen and metabolically active mucosal lamina propria predisposes epithelial cells to hypoxia-induced inflammation (Giatromanolaki et al., 2015). Concurrently, hypoxic brain tissues exhibit heightened proinflammatory cytokine release from the microglia, neurons, and astrocytes, substantially contributing to neural injury (Viviani et al., 2003).

In this study, the preventive effects of FCP may be attributed to its rich phenolic, flavonoid, and dietary fiber contents, collectively contributing to ROS scavenging (Meliala et al., 2024).

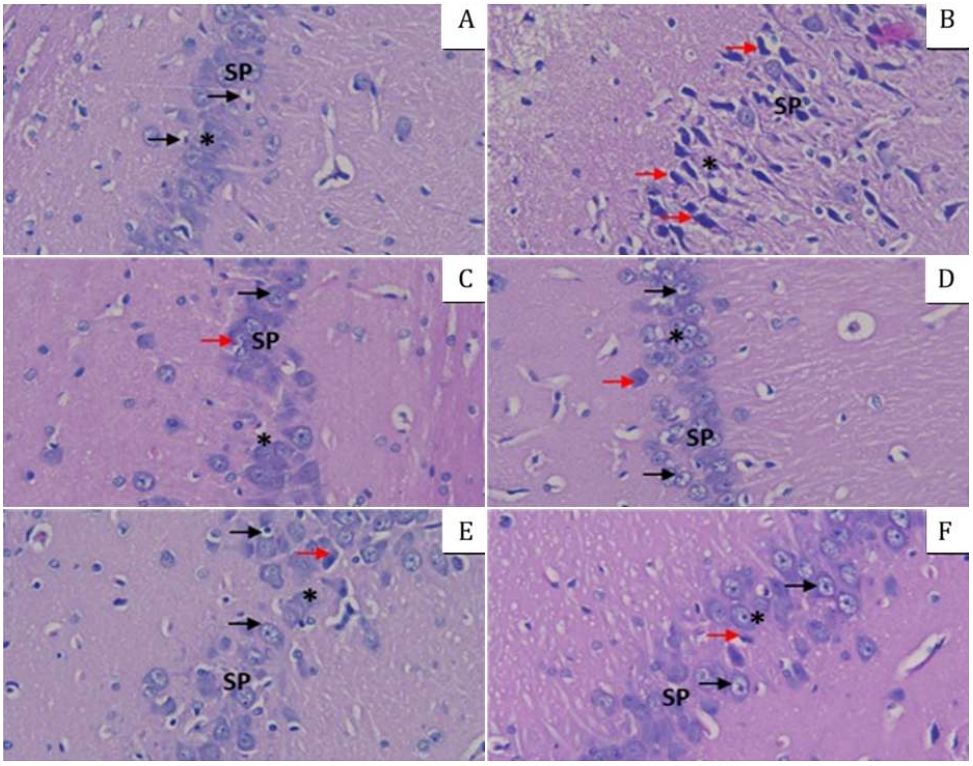


Figure 3. Representative Hematoxylin–eosin (HE) staining of rat hippocampal. Description: (→): Normal cell neuroglia, (→): Necrosis neuroglia cells A. Normoxia (NO), B. AH+corn oil 1mL/200g/weight/day (AHC), C. AH+Vit E 1mL/200g/weight/day (AHE), D. AH +FCP 1mL/200g/weight/day (AHF1), E. AH+FCP 2mL/200g/weight/day (AHF2), F. AH+FCP 4mL/200g/weight/day (AHF3). Cells of the stratum pyramidale (SP, asterisk) are shrinking compared to other groups. (A-F) 400X magnification.

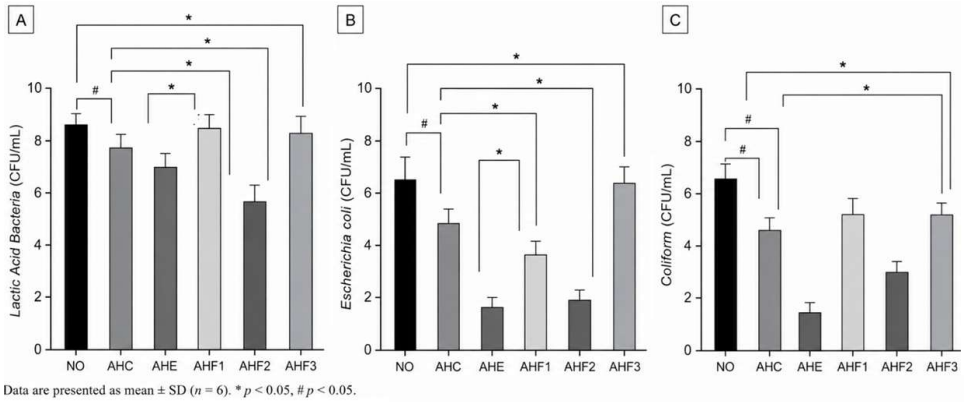


Figure 4. Total plate count (TPC) of bacterial colonies in cecal contents: (A) Lactic Acid Bacteria, (B) *Escherichia coli*, and (C) Coliforms. Experimental groups include NO (Normoxia control), AHC (Acute hypoxia + corn oil, 1 mL/200 g body weight/day), AHE (Acute hypoxia + vitamin E, 1 mL/200 g body weight/day), AHF1 (Acute hypoxia + *Ficus carica* puree [FCP], 1 mL/200 g body weight/day), AHF2 (Acute hypoxia + FCP, 2 mL/200 g body weight/day), and AHF3 (Acute hypoxia + FCP, 4 mL/200 g body weight/day). Statistical significance is indicated by * ($p < 0.05$; AHF1, AHF2, and AHF3 groups versus NO, AHC, and AHE groups) and # ($p < 0.05$; NO group versus AHC and AHE groups).

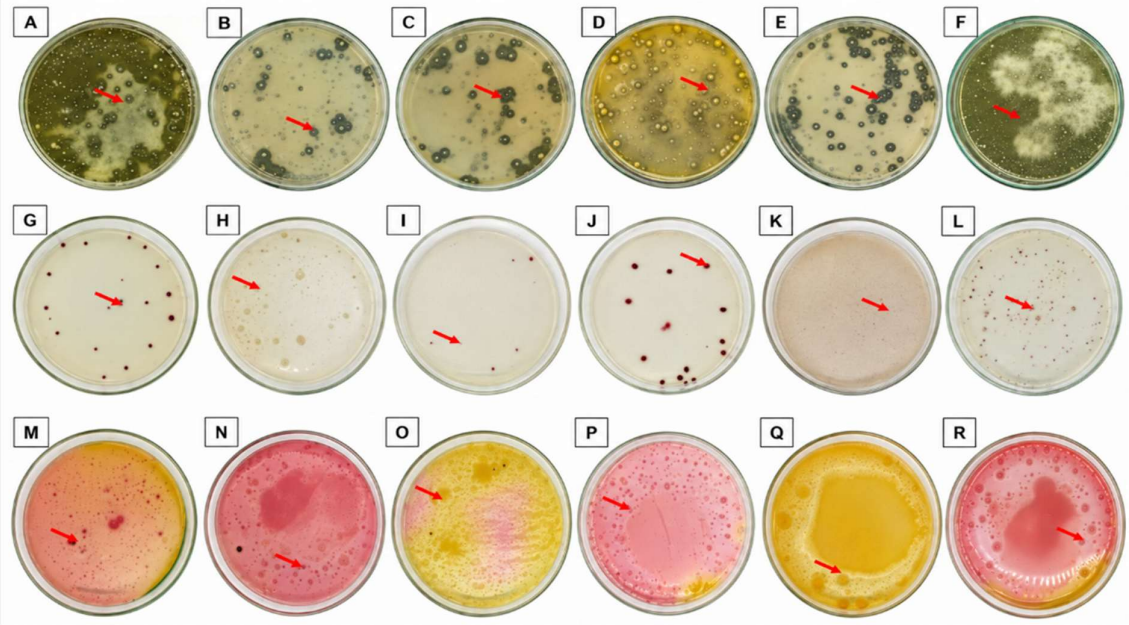


Figure 5. Representative images of total plate count (TPC) colonies from cecal contents. Arrow (→) indicates bacterial colonies. Panels (A–F) show lactic acid bacteria, (G–L) *Escherichia coli*, and (M–R) coliforms. Treatment groups are as follows: (A, G, M) Normoxia control (NO); (B, H, N) acute hypoxia + corn oil, 1 mL/200 g body weight/day (AHC); (C, I, O) acute hypoxia + vitamin E, 1 mL/200 g body weight/day (AHE); (D, J, P) acute hypoxia + *Ficus carica* puree (FCP), 1 mL/200 g body weight/day (AHF1); (E, K, Q) acute hypoxia + FCP, 2 mL/200 g body weight/day (AHF2); (F, L, R) acute hypoxia + FCP, 4 mL/200 g body weight/day (AHF3).

AH exposure for 4 h markedly suppressed the populations of LAB, *E. coli*, and coliforms in the AHC and AHE groups. Notably, only the AHF3 group, which received the highest FCP dose, consistently demonstrated elevated TPCs for LAB, *E. coli*, and coliforms (Figure 4). These findings align with those reported by Zou et al., who observed increased intestinal microbiome diversity and SCFA levels following dietary interventions (Zou et al., 2020). This finding suggests that the high dietary fiber content in FCP, particularly at a dose of 4 mL/200 g body weight, plays a pivotal role in modulating gut microbial populations and their metabolic activity. Future studies should focus on quantifying SCFA production in response to fluctuating FCP doses to better elucidate the dose-dependent effects.

We hypothesize that the antioxidative properties of FCP, coupled with its high fiber content, synergistically preserve intestinal barrier integrity and enhance SCFA synthesis through bacterial fermentation. The abundant fiber content in figs acts as a substrate for the gut microbiota, promoting SCFA production, which, in turn, exerts modulatory effects on brain function via the gut–brain axis (Dalile et al., 2019; Markowiak-Kope & Slizewska, 2020). Elevated SCFA levels can attenuate inflammation, reduce oxidative stress, and suppress ROS generation, thereby supporting neuronal health and cognitive function (Dalile et al., 2019; Markowiak-Kope & Slizewska, 2020; Zhang et al., 2023). Furthermore, gut microbiota influence the synthesis and expression of key neurotransmitters (e.g., serotonin and gamma-aminobutyric acid) and neurotrophic factors (e.g., brain-derived neurotrophic factor), while maintaining gut barrier function and regulating mucosal immunity (Carabotti et al., 2015).

Previous research has indicated that the antioxidant constituents of FCP effectively neutralize free radicals and suppress ROS and proinflammatory cytokine levels, thereby mitigating tissue damage in the brain and intestine (Eltzschig & Carmeliet, 2011; Mukandala et al., 2016; Rasool et al., 2023). Consistent with this, HE staining of the ileal tissue from the AHC group

revealed marked inflammatory cell infiltration in the lamina propria extending into the intestinal lumen, with some cells infiltrating epithelial crypts and evidence of epithelial cell degeneration. In contrast, the AHF1, AHF2, and AHF3 groups showed significantly reduced inflammatory cell infiltration, consistent with findings reported by Zou et al. (Zou et al., 2020).

Regarding hippocampal tissue, compared with the NO group, the AHC group exhibited marked lymphocyte infiltration, indicating an inflammatory response. Conversely, the FCP-supplemented groups displayed significantly reduced lymphocytic infiltration, highlighting the anti-inflammatory potential of FCP. These observations corroborate previous studies by Eteraf-Oskouei et al. and Jiang et al., demonstrating that fig supplementation attenuates inflammation through proinflammatory mediator downregulation (Eteraf-Oskouei et al., 2015; Jiang et al., 2019).

In this study, the observed neuroprotective effects against brain tissue damage may be mediated by the gut microbiome via the bidirectional gut–brain axis. This intricate communication network integrates the CNS, autonomic nervous system, enteric nervous system, and hypothalamic–pituitary–adrenal (HPA) axis, orchestrating systemic responses to environmental stressors (Tsigos & Chrousos, 2002).

The HPA axis, an essential component of the limbic system, is significantly involved in modulating memory, emotional processing, and the organism's adaptive stress response. Stress-induced elevation of systemic proinflammatory cytokine levels activates the limbic system, triggering hypothalamic secretion of corticotropin-releasing hormone, which, in turn, stimulates adrenocorticotropic hormone (ACTH) release from the pituitary gland. ACTH subsequently promotes cortisol secretion from the adrenal cortex, a glucocorticoid hormone that exerts extensive effects on multiple organs, including the brain and gastrointestinal tract. This neuroendocrine cascade emphasizes the significance of neural and humoral pathways in regulating intestinal functional effector cells, including immune cells, epithelial cells, enteric neurons, smooth muscle cells, and other components of the gut mucosa (Carabotti et al., 2015).

The antioxidative capacity of fig fruit is mainly attributed to its high phenolic, flavonoid, and dietary fiber contents, which collectively mitigate ROS overproduction, oxidative stress, and inflammation caused by AH. Fig fruit, as a functional food, facilitates the proliferation and maintenance of a healthy intestinal microbiome, thereby preserving mucosal integrity. Beyond its prebiotic benefits, FCP exerts direct antioxidant activity, contributing to the attenuation of inflammatory processes in ileal and cerebral tissues. These findings underscore the therapeutic potential of FCP in modulating gut–brain axis dynamics to confer systemic protection against hypoxia-induced oxidative and inflammatory damage.

This study mainly employed qualitative histological assessments to describe the morphological alterations in the hippocampus and ileum under hypoxic conditions. Figures 2 and 3 are designed to illustrate representative cellular changes, including neuronal shrinkage and mucosal disruption. Although a quantitative morphometric analysis (cell counting or percentage of shrunken cells) would improve the objectivity and reproducibility of the findings, the current study focused on descriptive histopathological observations, aligning with our primary aim to establish preliminary tissue-level protective effects of whole figs. To support and extend the qualitative observations presented here, future studies incorporating blinded quantitative scoring systems or image analysis software are warranted.

CONCLUSION

FCP effectively attenuates AH-induced ileal and hippocampal tissue damage by preserving gut microbiome integrity, thereby modulating gut–brain axis function. These findings highlight the potential of FCP as a functional intervention for protecting against hypoxia-related gastrointestinal and neurological dysfunction.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, in 2023 under the scheme of Penelitian Hibah Dana Masyarakat (No.: 692/UN1/FKKMK/PPKE/PT/ 2023). The remaining expenses, including laboratory materials and data analysis, were covered personally by the authors.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

ETHICAL APPROVALS

The research protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada-RSUP Dr. Sardjito (Approval No. KE/FK/0363/EC/2023; Date: 6 March 2023)

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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