

The effect of Sennoside A on the improvement of lipopolysaccharide -associated encephalopathy through gut microbiota

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Research

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Abstract

Background

Accumulating evidence suggests that the intestinal flora is involved in many neurodegenerative diseases. Sepsis can lead to severe intestinal flora imbalance and brain dysfunction. In this study, we investigated Sennoside A may relieve lipopolysaccharide(LPS)-associated encephalopathy via its effect on the gut microbiota in rats.

Methods

Adult male Sprague-Dawley (SD) rats and germ free (GF) rats were used. The ordinary and germ free SD rats were adopted as a LPS-associated encephalopathy model with or without Sennoside A administration. We investigated gut microbiota diversity and structure, conducted electroencephalograms (EEG) and measured the levels of TNF- α , IL-1 β and IL-6 in the cortexes of Sprague Dawley (SD) rats with or without Sennoside A administration. Horizontal fecal microbiota transplantation (FMT) and germ-free rats were used to confirm the important roles of gut microbiota in the mitigation of LPS-associated encephalopathy in rats after Sennoside A supplementation.

Results

We found that Sennoside A treatment markedly improved brain function in septic rats including decreased ratios of abnormal EEG and lowered levels of TNF- α , IL-1 β , and IL-6 in the rat cortexes. While the gut microbiota changed in septic SD rats, Sennoside A improved gut microbial composition, which might mediate its brain protective effects in sepsis. Sennoside A also reduced inflammation in the cortexes of septic rats via gut microbiota improvement. In germ-free rats that received lipopolysaccharide(LPS), Sennoside A could not lower the ratios of abnormal EEG, and could not alleviate TNF- α , IL-1 β , and IL-6 levels in the rats' cortexes. FMT lowered the ratios of abnormal EEG and alleviate TNF- α , IL-1 β , and IL-6 levels in rats' cortexes, which confirmed our hypothesis that the effect of Sennoside A on the improvement of LPS-associated encephalopathy through gut microbiota.

Conclusion

Our data confirm our hypothesis that Sennoside A likely exerts its brain protective effects through gut microbiota alteration.

Introduction

Sepsis is one of the leading causes of morbidity and mortality in patients in critical care and acute care wards^[1]. Sepsis-induced brain dysfunction is called sepsis-associated encephalopathy (SAE), the

mechanisms underlying these complications remain unclear and an effective intervention is lacking^[2]. Thus, exploring the pathogenesis of SAE is important to develop more effective prevention and treatment strategies.

Accumulating evidence suggests that the intestinal flora is involved in many neurodegenerative diseases. Ma et al. discovered *Bifidobacterium infantis* M-63 elevated mental health in individuals with irritable bowel syndrome that developed after a major flood disaster^[3]. Lowe et al. found that acute-on-chronic alcohol administration induces alcohol-associated central nervous system and gut inflammation, which could be reduced by gut microbiome intervention^[4]. A previous study from our group proved that modulation of the intestinal microbiota in septic rats could alleviate SAE^[5].

Sepsis can lead to severe intestinal flora imbalance. Chen discovered that the structure and composition of intestinal flora change significantly in rats with sepsis^[6]. Gong determined that gut microbiota imbalance was an important mediator of sepsis-induced liver injury^[7]. Beckmann found that burn injury patients had a high risk of sepsis-related mortality, and these patients had significant changes in their intestinal microbiome composition^[8]. To Modulating this microbiota dysbiosis may be a potential method to improve SAE in septic rats.

Therefore, targeting the gut microbiota with appropriate drugs is hypothesized to be an effective therapy for SAE. Sennoside A, an inactive glycoside in rhubarb, is the major purgative component of rhubarb^[9]. Rhubarb is a common traditional Chinese medicine used to regulate enteric function. It is known that rhubarb can rectify an intestinal flora imbalance^[10]. Yao discovered that rhubarb can rectify intestinal flora in rats with severe acute pancreatitis^[11]. The anti-tumor properties of rhubarb have stimulated research to assess its effect in shaping the gastrointestinal bacterial diversity of young animals when used as a feed additive^[10]. Accumulating evidence supports the finding that Sennoside A and rhubarb can alter intestinal bacterial composition and hence exert multiple pharmacological effects^[12]. Nevertheless, it remains unknown whether Sennoside A affects LPS-associated encephalopathy via the gut microbiota. Fecal microbiota transplantation (FMT), as an effective methods to improve the dysbiosis of intestinal microbiota, is an effective method in SAE treatment^[5].

To test the hypothesis that gut microbiota alteration may be involved in the pathogenesis of LPS-associated encephalopathy, and may be ameliorated by Sennoside A, LPS-induced sepsis was used, LPS can make "model of acute inflammation", which can be used to study sepsis^[13]. Ordinary and germ free SD rats were adopted as a model. The ratio of abnormal EEGs, levels of TNF- α , IL-1 β , and IL-6 in the rat cortexes were assessed after Sennoside A administration or FMT. The effects of Sennoside A on intestinal microbiotas were evaluated by analyzing the bacterial V3-V4 regions of 16S rRNA genes by Illumina sequencing and multivariate statistical analysis.

Materials And Methods

Animals

Eighty adult male Sprague-Dawley (SD) rats and forty germ free (GF) rats were purchased from Hebei Medical University, Shijiazhuang, China. The protocol was approved by the Ethics Committee of Hebei General Hospital, and all procedures were performed in accordance with the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health, USA. The animals were housed under a 12-h light/dark cycle in a temperature-controlled room at $24\pm 1^{\circ}\text{C}$ with free access to food and water. Germ free rats were given free access to germ free food and water (all food, water, bedding and other supplies that were put in the isolator were sterilized in advance)^[14].

Experimental procedures

Eighty ordinary SD rats were randomly divided into four equal groups: Sham treatment group, LPS group (LPS+saline), LPS with Sennoside A treatment group (LPS+SA), LPS with FMT treatment group (LPS+FMT). Germ free SD rats were randomly divided into two groups: rats given LPS (LPS+GF+saline), and rats given LPS and Sennoside A treatment (LPS+GF+SA). In the LPS+SA group, rats were gavaged with Sennoside A at $25\text{mg}\cdot\text{kg}^{-1}$ every 8 hours; in the sham and LPS +saline groups, rats were given normal saline instead of Sennoside A. For the rats in LPS +FMT group, fecal microbiota transplantation was performed with fresh feces from the healthy donor rats three times a day for 7 days. Germ free rats in the LPS+GF+SA group were gavaged with Sennoside A at $25\text{mg}\cdot\text{kg}^{-1}$ every 8 hours. In the LPS+GF+saline group, rats were given normal saline instead of Sennoside A. Sennoside A and saline were sterilized in advance. Rats in all groups except the sham group received an intravenous injection of $10\text{mg}/\text{kg}$ body weight lipopolysaccharide(LPS from *Escherichia coli*, O111:B4; Sigma-Aldrich Chemie GmbH, Germany) through the caudal vein, while rats in the sham group were given the same volume of saline. After the 7th day, fecal samples were collected, and 1g of each sample was immediately stored at -80°C until DNA extraction and subsequent use to study microbiota composition with 16S rDNA analysis.

Enzyme-linked immunosorbent (ELISA) assay

The brains were removed, stored at -80°C ; the brain cortex samples were collected for TNF- α , IL-1 β , and IL-6 detection 48h after LPS or saline administration. The concentrations of TNF- α , IL-1 β , and IL-6 were detected by ELISA kits according to the manufacturer's instructions. Standard curves were constructed using various dilutions of manufacturer-supplied TNF- α , IL-1 β , and IL-6 standards. The levels of the cytokines were calculated according to the standard curves.

PCR and sequence analysis

Fecal samples were harvested and used for bacterial DNA extraction and sequencing of the V3-V4 hypervariable region in the 16S rDNA gene.

EEG Recordings and analysis

EEGs were recorded at 48h after LPS or saline administration. Standard EEG was performed using a Nihon Kohden manufactured EEG-9100J/K portable digital EEG system. EEG recordings and analysis followed the guidelines of the International Federation of Clinical Neurophysiology.

Statistics analysis

All data are presented as mean±standard error of the mean (SEM) or standard deviation (SD). Statistical analyses were performed using a one-way analysis of variance. In cases of significance, a Fisher post hoc test was applied (Statview, SAS, Cary, NA, USA). Correlation between two variances was estimated using linear regression analysis with a Pearson test. The significance level was set to $P<0.05$.

Results

1. Gut microbiota analysis

1.1 The diversity of the fecal microbiota

The fecal microbiota diversity in the four groups of SD rats was assessed on the 7th day based on the Chao1 index and observed species richness. The LPS+saline group had significantly lower phylogenetic diversity compared to the sham group, indicating a less diverse fecal microbial composition. The LPS+SA and the LPS+FMT groups had approximately equal phylogenetic diversities compared to the sham group on the 7th day post Sennoside A administration and FMT treatment (FIG.1a, FIG.1b). These data indicated that LPS significantly impacted the richness of the gut microbiota, and that Sennoside A and FMT can protect this gut microbial diversity in the LPS induced septic rats.

2 The relative abundances of the fecal microbiota

Analyses of microbiomes showed shifts in bacterial relative abundances upon FMT and Sennoside A supplementation. As illustrated FIG.2a, at the phylum level, the most abundant microbiota in the gut of LPS-treated rats were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. The microbiome composition was altered in the LPS+saline group: there was significantly greater abundance of *Proteobacteria* and significantly lower abundance of *Firmicutes* compared with the sham group. Meanwhile, the abundance of *Bacteroidetes* was decreased, whereas *Actinobacteria* was almost unchanged. These values reflect the percentage of the mean relative abundance of this bacterial group in the four groups (not including the germ free groups). Sennoside A supplementation and FMT were found to markedly modify relative percentages of detected phyla when compared to the LPS+saline rats. Sennoside A and FMT supplementation were able to marginally ($P=0.00$, $P=0.00$) increase *Firmicutes*, and *Bacteroidetes*. Bacterial community composition was similar in Sennoside A and FMT groups compared to the sham group.

As illustrated FIG.2b, at the family level, in the LPS+saline rats, the mean relative abundances of *Ruminococcaceae*, *Clostridiaceae*, *Lactobacillaceae*, *Erysipelotrichaceae*, *Bacteroidaceae*, and *Eubacteriaceae*, were significantly reduced compared to the sham-treated rats, meanwhile, the mean relative abundances of *Lachnospiraceae*, *Prevotellaceae*, *Graciibacteraceae*, *Acidaminococcaceae*, and *Acholeplasmataceae* were significantly increased compared to the sham-treated rats. The mean relative abundances of *Ruminococcaceae*, *Clostridiaceae*, and *Lachnospiraceae* were the most affected bacterial groups after Sennoside A supplementation and FMT; the mean relative abundance of *Ruminococcaceae*

and *Clostridiaceae* were significantly increased compared to the LPS+saline rats, and the mean relative abundance of *Lachnospiraceae* was significantly decreased compared to the LPS+saline rats.

Regarding the genus level, LPS+saline rats had significantly reduced levels of *Faecalibacterium* when compared to sham-treated rats. In contrast, LPS+saline rats had significantly increased levels of *Alistipes*, compared to the sham group. Sennoside A and FMT treatments significantly increased the levels of *Faecalibacterium* when compared to LPS+saline rats, and reduced the abundance of *Alistipes* compared to the sham group (FIG.3a,3b).

3. Analysis of TNF- α , IL-1 β , and IL-6 levels in rat cortex.

SAE rats usually present with higher levels of inflammatory cytokines, including TNF- α , IL-1 β , and IL-6 in cortex tissues^[5]. We measured the levels of these cytokines after the 7th day of Sennoside A feeding. We determined that the levels of these cytokines were lower in the cortex tissues of Sennoside A-fed rats. More interestingly, germ-free rats that received the LPS and Sennoside A, did not reduce TNF- α , IL-1 β , and IL-6 levels in cortex tissues (FIG.4a,4b,4c). These results showed that the supplementation of Sennoside A significantly lowered the levels of TNF- α , IL-1 β , and IL-6 in the cortex through gut microbiota alteration ($P < 0.05$) (FIG.4a,4b,4c).

4. Sennoside A improved LPS-associated encephalopathy

Abnormal EEGs indicate the degree of SAE^[13]. EEGs showed that in rats treated with LPS there was a remarkable increase of reactivity, excessive theta and delta activity, electrographic seizures (ESZ) and periodic discharges (PD) compared with the sham-treated group. However, these abnormalities were reduced in the groups of rats treated with Sennoside A and FMT. These results suggest that Sennoside A improves brain function of rats with sepsis (Table1). Thus, both Sennoside A and FMT relieved inflammation in the cortex tissues of rats with sepsis.

To further confirm the hypothesis that SAE was affected by the gut microbiome, germ free rats were studied. The ratios of abnormal EEGs were not improved after Sennoside A therapy in germ free rats (Table1). In short, Sennoside A likely exerts its brain protective effects through gut microbiota alteration.

Discussion

This study demonstrated that Sennoside A treatment improved the dysbiosis of intestinal microbiota in LPS-associated encephalopathy rats. Sennoside A supplementation had a substantial impact on gut microbiota diversity and composition at different taxonomic levels. We were particularly interested in the abundance of bacterial species such as *Faecalibacterium* and *Alistipes*, which had previously been associated with brain function. The incidence of abnormal EEGs, the cortex levels of TNF- α , IL-1 β , and IL-6, were also significantly decreased in the Sennoside A group compared with the LPS group.

More interestingly, in GF rats, Sennoside A did not lower the ratio of abnormal EEG, or lower TNF- α , IL-1 β , and IL-6 levels in rat cortexes. Abnormal EEG and elevated inflammatory mediators indicate the degree of SAE^[14]. The present results support the hypothesis that gut microbiota is involved in the pathogenesis of

LPS-associated encephalopathy and that Sennoside A exerts its brain protective effects through gut microbiota alteration.

Other authors have also shown that Sennoside A, the main glycoside in rhubarb, can normalize the gut microbiota. Wei et al., found that Sennoside A could effectively improve the abundance of the intestinal flora, and alleviate Type 2 diabetes symptoms and reduce obesity^[15]. Sennoside A restored the gut microbiota profile, increased short chain fatty acids (SCFAs), improved mucosal structure in the colon and restored the function of the microbiota-GLP1 axis to improve glucose metabolism in obese mice^[16]. Yao et al., found that rhubarb supplementation increased the abundance of intestinal lactobacilli and bifidobacteria, and decreased the amount of intestinal *Escherichia coli*. In rats with severe acute pancreatitis^[11]. These findings are consistent with our study. Sennoside A exerted its corrective effects at phylum and genus levels, especially affecting the abundance of *Faecalibacterium* and *Alistipes*, which play key roles in brain function. Numerous studies showed that FMT is an effective method to modulate the dysbiosis of intestinal microbiota in many diseases^[5, 17]. This study showed that Sennoside A and FMT have similar effects in modulating the dysbiosis of intestinal flora in septic rats.

Previous human studies investigated the influence of the intestinal microbiota on the brain and behavior, which is referred to as the gut-brain axis. Han et al., demonstrated decreased β diversity and intestinal microbiota richness (including Faecalibaculum) that led to cognitive dysfunction after surgery in APP/PS1 mice; however, probiotics' alteration of the intestinal microbiota attenuated this cognitive dysfunction^[18]. Alterations in gut microbial composition have also been observed in children with autism spectrum disorder (ASD), with a decrease in the *Firmicutes/Bacteroidetes* ratio^[19] compared to children without ASD. Depression was negatively associated with *Lachnospiraceae* abundance. Patients with anxiety were characterized by elevated *Bacteroidaceae*^[20]. Rifaximin administration is associated with microbial changes, including an increase in *Eubacteriaceae* and a decrease in *Veillonellaceae* abundance, which is accompanied by improved cognitive function in minimal hepatic encephalopathy^[21]. Nogay et al., demonstrated that high growth rates of *Clostridium histolyticum*, *C. perfringens*, and *Sutterella*, high ratios of *Escherichia/Shigella*, and low ratios of *Bacteroidetes/Firmicutes* were generally related to GI problems, while relative abundance of *Desulfovibrio*, *Clostridium* spp., and *Bacteroides vulgatus* were associated with behavioral disorders. However, the available information is not yet detailed enough to develop a gut microbiota-based nutritional intervention to treat GI symptoms in people with autism^[22]. *Alistipes* was over-represented in patients with depression, an increase in *Alistipes* was observed in chronic social stress and was correlated with increases in proinflammatory cytokines IL-6 and TNF- α ^[23]. Notably, enrichment of *Alistipes* and reduction of *Faecalibacterium*, was shown to be associated with brain injury^[24]. The genus *Faecalibacterium* is populated by bacterial species that produce SCFAs, which have an anti-inflammatory effect and are therefore beneficial to the host. Butyrate-producing *Faecalibacterium* and *Coprococcus* bacteria were consistently associated with higher quality brain function, and are implicated in relieving alcohol-induced anxiety^[25]. This study proved that Sennoside A supplementation can correct the dysbiosis of intestinal flora structure in sepsis, especially the abundance of bacteria closely related to brain function, which may be a key reason why treatment with Sennoside A played an effective role in alleviating SAE symptoms.

The main pathogenesis of SAE is inflammation in the brain, including increased levels of TNF- α , IL-1 β , and IL-6 in the cortex. Abnormal EEG can be used to diagnose SAE^[26]. Evaluation of microbiota diversity indicated that a reduced microbial diversity is associated with inflammation, and changes in the relative abundance of *Firmicutes* and *Bacteroidetes* have been determined to affect the balance of inflammation. In particular, a rise in the *Firmicutes/Bacteroidetes* ratio is related to low-grade inflammation^[27]. Reductions in the abundance of certain microbes, such as *Faecalibacterium*, which produce SCFAs, were associated with higher levels of inflammation in inflammatory bowel disease and autoimmune diseases^[28]. Moreover, *Faecalibacterium* produces a 15kDa anti-inflammatory protein that inhibits the NF- κ B pathway in intestinal epithelial cells and was shown to decrease the level NF- κ B, IL-1 β , IL-6 and TNF- α in a mouse model^[29]. In addition, a rise in *Alistipes* has previously been observed in chronic social stress, and is correlated with increases in proinflammatory cytokines IL-6 and TNF- α ^[30]. This study showed that Sennoside A supplementation decreased the levels of IL-6, TNF- α , and IL-1 β in SD rats' cortexes. In germ-free rats that received LPS, Sennoside A supplementation did not lower the ratios of abnormal EEGs, nor lower TNF- α , IL-1 β , and IL-6 levels in rats' cortex. Sennoside A likely exerted its brain protective effects through gut microbiota alteration.

Conclusions

In summary, the present study demonstrated that Sennoside A supplementation and FMT can improve the diversity of SD rats' intestinal microbiota, and promotion of "beneficial bacteria" improved brain function. In short, Sennoside A can be used as a microbial regulator to improve LPS-associated encephalopathy. Sennoside A likely exerted its brain protective effects through gut microbiota alteration.

Declarations

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Authors' contributions

Fenyan Zhang participated in the research design, experimental performance (including animal surgery), but not the data analysis, and drafting of the manuscript. Yiguang Lin provided technical assistance and help with manuscript preparation. Xiaoli Niu and Jian Lv, and Ranliang Hua discussed the results and edited parts of manuscript. Qian Zhao and Liru Zhang, performed injection, and data analysis. Hui Guo participated in the research design. Suyan Li are the corresponding authors, participated in all aspects of the study, including research design, data analysis, and manuscript preparation. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate

All animal experimental protocols were approved by the Ethics Committee of Hebei General Hospital, and all procedures were performed in accordance with the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health, USA.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest. All the authors listed have approved the manuscript.

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Abbreviations

FMT: Fecal microbiota transplantation; LPS: Lipopolysaccharide; SAE: Sepsis-associated encephalopathy; TNF α : Tumor necrosis factor- α ; IL-1 β : Interleukin-1 β ;IL-6: Interleukin-6; EEG: Electroencephalogram; ESZ:electrographic seizures; PD:Periodic discharges; SCFAs:Short chain fatty acids; ASD:Autism spectrum disorder.

References

- 1 Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med*. 2013. 369(9): 840-51.
- 2 Hazama K, Shiihara T, Tsukagoshi H, Matsushige T, Dowa Y, Watanabe M. Rhinovirus-associated acute encephalitis/encephalopathy and cerebellitis. *Brain Dev*. 2019. 41(6): 551-554.
- 3 Ma ZF, Yusof N, Hamid N, et al. Bifidobacterium infantis M-63 improves mental health in victims with irritable bowel syndrome developed after a major flood disaster. *Benef Microbes*. 2019. 10(2): 111-120.
- 4 Lowe PP, Gyongyosi B, Satishchandran A, et al. Reduced gut microbiome protects from alcohol-induced neuroinflammation and alters intestinal and brain inflammasome expression. *J Neuroinflammation*. 2018. 15(1): 298.
- 5 Li S, Lv J, Li J, et al. Intestinal microbiota impact sepsis associated encephalopathy via the vagus nerve. *Neurosci Lett*. 2018. 662: 98-104.
- 6 Chen L, Li H, Li J, Chen Y, Yang Y. Lactobacillus rhamnosus GG treatment improves intestinal permeability and modulates microbiota dysbiosis in an experimental model of sepsis. *Int J Mol Med*. 2019. 43(3): 1139-1148.
- 7 Gong S, Yan Z, Liu Z, et al. Intestinal Microbiota Mediates the Susceptibility to Polymicrobial Sepsis-Induced Liver Injury by Granisetron Generation in Mice. *Hepatology*. 2019. 69(4): 1751-1767.
- 8 Beckmann N, Pugh AM, Caldwell CC. Burn injury alters the intestinal microbiome's taxonomic composition and functional gene expression. *PLoS One*. 2018. 13(10): e0205307.
- 9 Malik EM, Müller CE. Anthraquinones As Pharmacological Tools and Drugs. *Med Res Rev*. 2016. 36(4): 705-48.
- 10 Huang Q, Lu G, Shen HM, Chung MC, Ong CN. Anti-cancer properties of anthraquinones from rhubarb. *Med Res Rev*. 2007. 27(5): 609-30.
- 11 Yao P, Cui M, Li Y, Deng Y, Wu H. Effects of rhubarb on intestinal flora and toll-like receptors of intestinal mucosa in rats with severe acute pancreatitis. *Pancreas*. 2015. 44(5): 799-804.
- 12 Wang J, Zhao H, Kong W, et al. Microcalorimetric assay on the antimicrobial property of five hydroxyanthraquinone derivatives in rhubarb (*Rheum palmatum* L.) to *Bifidobacterium adolescentis*. *Phytomedicine*. 2010. 17(8-9): 684-9.
- 13 Osuchowski MF, Ayala A, Bahrami S, et al. Minimum quality threshold in pre-clinical sepsis studies (MQTiPSS): an international expert consensus initiative for improvement of animal modeling in sepsis. *Intensive Care Med Exp*. 2018. 6(1): 26.
- 14 Qv L, Yang Z, Yao M, et al. Methods for Establishment and Maintenance of Germ-Free Rat Models. *Front Microbiol*. 2020. 11: 1148.

- 15 Wei Z, Shen P, Cheng P, Lu Y, Wang A, Sun Z. Gut Bacteria Selectively Altered by Sennoside A Alleviate Type 2 Diabetes and Obesity Traits. *Oxid Med Cell Longev*. 2020. 2020: 2375676.
- 16 Le J, Zhang X, Jia W, et al. Regulation of microbiota-GLP1 axis by sennoside A in diet-induced obese mice. *Acta Pharm Sin B*. 2019. 9(4): 758-768.
- 17 Baruch EN, Youngster I, Ben-Betzalel G, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*. 2021. 371(6529): 602-609.
- 18 Han D, Li Z, Liu T, et al. Prebiotics Regulation of Intestinal Microbiota Attenuates Cognitive Dysfunction Induced by Surgery Stimulation in APP/PS1 Mice. *Aging Dis*. 2020. 11(5): 1029-1045.
- 19 Strati F, Cavalieri D, Albanese D, et al. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome*. 2017. 5(1): 24.
- 20 Peter J, Fournier C, Durdevic M, et al. A Microbial Signature of Psychological Distress in Irritable Bowel Syndrome. *Psychosom Med*. 2018. 80(8): 698-709.
- 21 Bajaj JS, Heuman DM, Sanyal AJ, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One*. 2013. 8(4): e60042.
- 22 Nogay NH, Nahikian-Nelms M. Can we reduce autism-related gastrointestinal and behavior problems by gut microbiota based dietary modulation? A review. *Nutr Neurosci*. 2019 : 1-12.
- 23 Bangsgaard Bendtsen KM, Krych L, Sørensen DB, et al. Gut microbiota composition is correlated to grid floor induced stress and behavior in the BALB/c mouse. *PLoS One*. 2012. 7(10): e46231.
- 24 McIntosh CM, Chen L, Shaiber A, Eren AM, Alegre ML. Gut microbes contribute to variation in solid organ transplant outcomes in mice. *Microbiome*. 2018. 6(1): 96.
- 25 Valles-Colomer M, Falony G, Darzi Y, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat Microbiol*. 2019. 4(4): 623-632.
- 26 Shulyatnikova T, Verkhatsky A. Astroglia in Sepsis Associated Encephalopathy. *Neurochem Res*. 2020. 45(1): 83-99.
- 27 Pascale A, Marchesi N, Govoni S, Coppola A, Gazzaruso C. The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases. *Curr Opin Pharmacol*. 2019. 49: 1-5.
- 28 Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ*. 2018. 360: j5145.
- 29 Quévrain E, Maubert MA, Michon C, et al. Identification of an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in Crohn's disease. *Gut*. 2016. 65(3): 415-425.

30 Belenguer A, Duncan SH, Calder AG, et al. Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microbiol.* 2006. 72(5): 3593-9.

Tables

Table 1. The proportion of abnormal EEG results in each group

Variables	Sham	LPS+Saline	LPS+FMT	LPS+SA	LPS+GF+Saline	LPS+GF+SA
Delta-predominant	0(20)	8(9) #	6(13) #*	6(12) #*	8(8)	7(8)
Theta-predominant	0(20)	7(9) #	3(13) #*	2(12) #*	6(8)	6(8)
Low voltage	0(20)	8(9) #	2(13) #*	3(12) #*	6(8)	7(8)
Absence of reactivity	0(20)	8(9) #	1(13) #*	1(12) #*	7(8)	6(8)
Electrographic seizure	0(20)	6(9) #	2(13) #*	1(12) #*	5(8)	6(8)
Triphasic waves	0(20)	4(9) #	0(13) #*	1(12) #*	3(8)	4(8)
Periodic Discharges	0(20)	2(9) #	2(13) #*	1(12) #*	4(8)	3(8)

p < 0.05. # compared with Sham group, * compared with LPS+Saline group, □ compared with LPS+GF+Saline group.

Figures

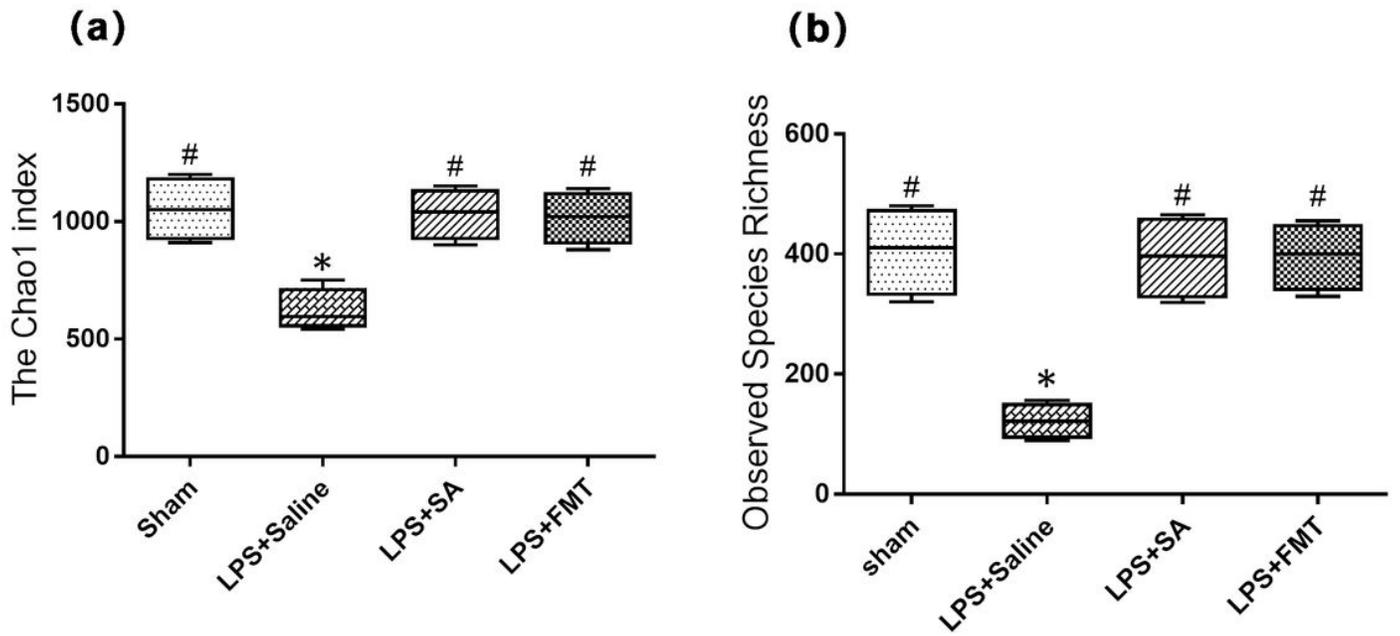


Figure 1

Sennoside A and FMT alters the diversity of the fecal microbiota in animals with sepsis. Changes in (a) the Chao1 index, and (b) observed species richness. Data are presented Mean \pm SD.

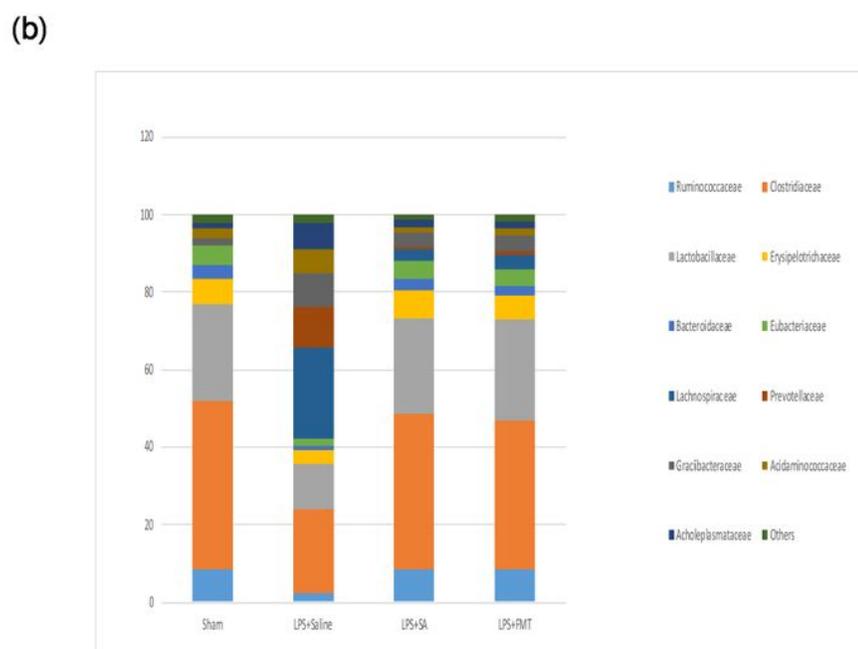
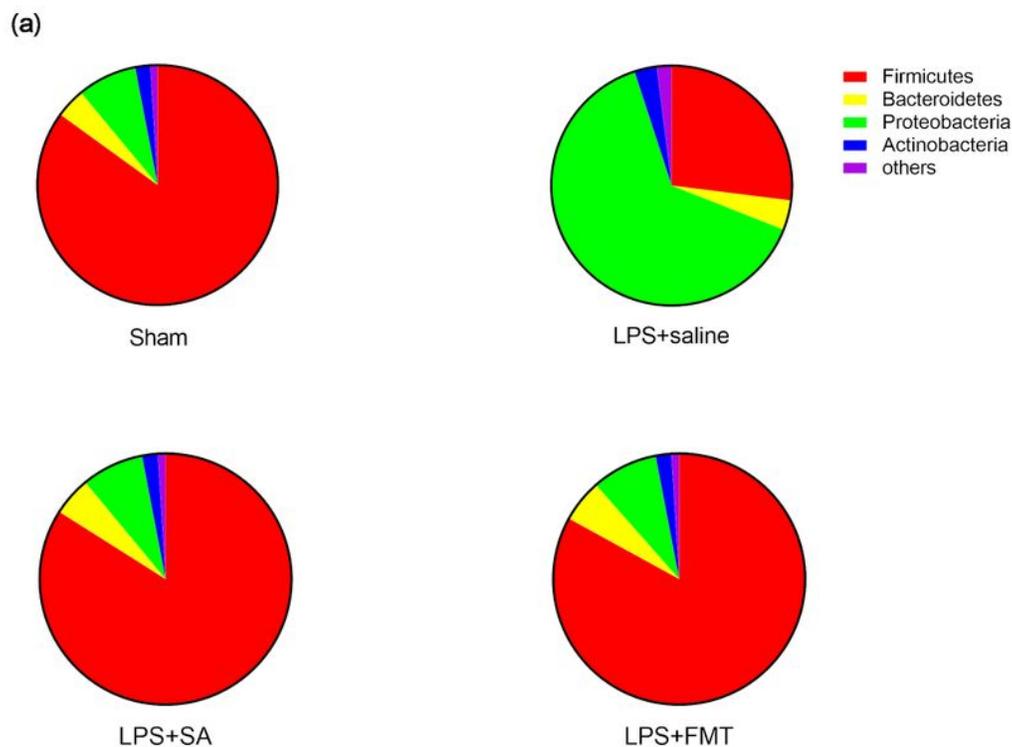


Figure 2

Phylum-level distributions(a) and family-level taxonomic distributions (b) of the microbial communities in faeces contents. Rats in sham group (n=20) , Rats in LPS+saline group (n=9) , Rats in LPS+SA group (n=12), Rats in LPS+FMT group (n=12).

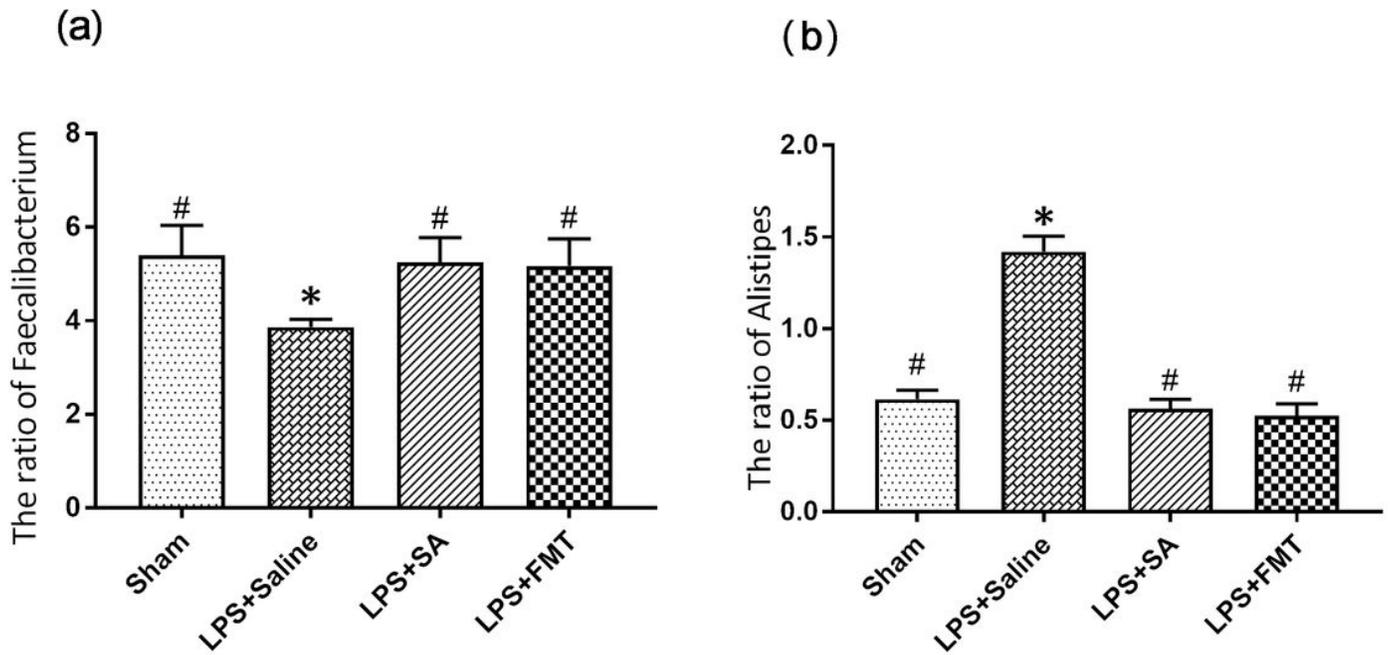


Figure 3

The ratio of Faecalibacterium(a) and (b) Alistipes in faeces contents. #P compared with LPS+saline group, $P < 0.05$. *P compared with sham group, $P < 0.05$.

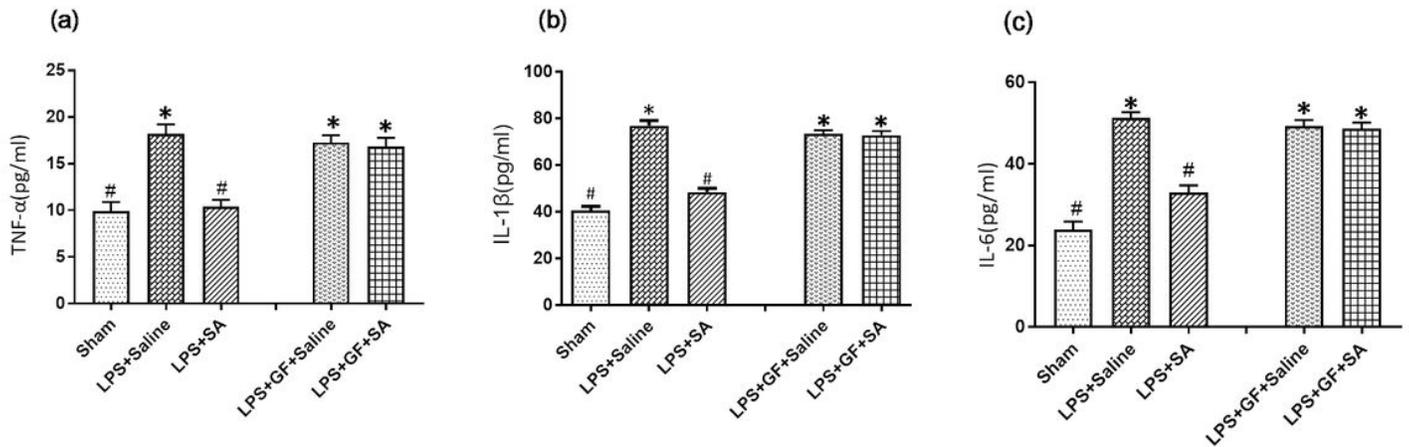


Figure 4

The level of TNF- α (a) ,IL-1 β (b), IL-6(c) in faeces. Rats in sham group (n=20), Rats in LPS+saline group (n=9), Rats in LPS+SA (n=12), Rats in LPS+GF+Saline group (n=9), Rats in LPS+GF+SA group (n=9). #P compared with LPS+saline group, $P < 0.05$. *P compared with sham group, $P < 0.05$. \boxtimes P compared with LPS+GF+Saline sham group, $P < 0.05$.