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Dietary Supplement of *Anoectochilus Roxburghii (Wall.) Lindl* Polysaccharides Ameliorates Cognitive Dysfunction induced by high fat diet via “Gut-Brain” axis

Liya Fu
The Affiliated Hospital of Southwest Medical University
Wanlong Zhu
The Affiliated Hospital of Southwest Medical University
Dongmei Tian
The Affiliated Hospital of Southwest Medical University
Yong Tang
Southwest Medical University, Luzhou Sichuan, China
Yun Ye
The Affiliated Hospital of Southwest Medical University
Qiming Wei
The Affiliated Hospital of Southwest Medical University
Chengbin Zhang
the Affiliated Hospital of Southwest Medical University
Wenqiao Qiu
Luzhou Medical College: Southwest Medical University
Dalian Qin
Luzhou Medical College: Southwest Medical University
Xuping Yang (yangxuping6552@163.com)
The Affiliated Hospital of Southwest Medical University
Yilan Huang (lzyxyyxp123@163.com)
The Affiliated Hospital of Southwest Medical University

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Abstract

Purpose *Anoectochilus roxburghii (Wall.) Lindl* polysaccharides (ARPs) has been reported to exhibit multiple pharmacological activities including anti-inflammatory and anti-hyperglycemia. The study tried to investigate the effect of ARPs on cognitive dysfunction induced by high fat diet (HFD).

Methods C57BL/6J mice were given ARPs by dietary supplementation for 14-week. The effect of ARPs on cognitive function was determined by assessing the changes in spatial learning and memory ability, neurotrophic factors in hippocampus, inflammatory parameters, intestinal barrier integrity, and fecal microbiota.

Results ARPs supplementation can effectively ameliorate cognitive dysfunction, decrease the expressions of Tau protein in hippocampus. Meanwhile, increased body weight, plasma glucose and lipids levels induced by HFD were abolished by ARPs treatment. It was revealed that the levels inflammatory cytokines and the LPS were remarkably higher in the HFD group than those in the chow diet group, but ARPs treatment reversed these detrimental effects. Furthermore, ARPs treatment restored the intestinal epithelial barrier as evidenced by upregulation of intestinal tight junction proteins (ZO-1 and occluding). Additionally, ARPs supplementation significantly decreased the relative abundance of eight bacteria genus such as *Parabacteroides*, which was negatively correlated with cognitive function.

Conclusions These results suggest that ARPs might be a promising strategy for the treatment of cognitive dysfunction induced by HFD. Mechanistically, alleviation of cognitive dysfunction by ARPs was at least partially through regulating the “gut-brain” axis.

Introduction

Cognitive dysfunction is a syndrome manifested primarily by impairment of learning and memory functions[1]. Numerous researches suggest obesity or over-weight are associated with deficits in learning, memory, executive function as well as potential brain atrophy[2]. Inflammation is considered to be involved in the pathophysiology of cognitive impairment and dementia, which is associated with the neuropathological features of Alzheimer’s disease[3]. Multiple studies including obese subjects have shown that systemic inflammation is positively associated with symptoms of cognitive decline[4]. Neuroinflammation promotes central insulin resistance, which ultimately leads to hyperphosphorylation of Tau protein, inducing hippocampal neuronal apoptosis[5]. Thus, ameliorating obesity and the related inflammation may have a beneficial impact on cognitive function.

It is well documented that changes in intestinal microbiota can modulate the peripheral and central nervous systems, influencing brain function via a pathway called “gut-brain” axis[6-8]. A review by Chang *et al.* reported that the conversion of L-glutamate to D-glutamate by *Bacteroides* and other bacterial species may affect cognitive function in Alzheimer’s disease, which supports the importance of intestinal

bacteria metabolism on cognitive function and memory[9]. Meanwhile, metabolic diseases such as type 2 diabetes or fatty liver disease are closely related to the gut microflora dysbiosis[10]. Long-term high-fat diet not only promotes changes in the composition of intestinal microflora and metabolites, but also leads to extensive accumulation of lipids in intestinal cells, oxidative stress, and damaged intestinal barrier[7]. In obese individuals, large quantities of lipopolysaccharides (LPS) in intestinal enter the blood circulation and stimulate the secretion of pro-inflammatory factors, which trigger or intensify neuroinflammation and aggravates cognitive dysfunction[3, 5].

It has been shown that some herbal prescriptions and monomers such as *ginkgo biloba* preparations and Luteolin can protects against high fat diet-induced cognitive deficits in obesity mice [11-13]. *Anoectochilus roxburghii (Wall.) Lindl* is a tranditional Chinese medicine, *Anoectochilus roxburghii (Wall.) Lindl* polysaccharides (ARPs) is one of the main components with multiple pharmacological activities including anti-oxidant, and anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, anti-bacterial, anti-tumor[14, 15]. Liu *et al*. found that ARPs improves obesity-associated inflammation by regulating the p38 mitogen-activated protein kinase (p38/MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathways[16]. Moreover, Wang et al. showed that *Anoectochilus roxburghii (Wall.) Lindl* extract significantly delayed aging in a D-galactose-induced dementia model[17]. Another study showed that ARPs lowered the pH value in the cecum and increased its content of probiotic *bifidobacterial[18]*. Therefore, we speculate that ARPs may ameliorate inflammation and cognitive dysfunction induced by HFD. In this study, we constructed a diet-induced-obese mouse model and investigated the effects of ARPs on cognitive function and the related mechanisms.

Materials & Methods

ARPs extraction

Anoectochilus roxburghii (Wall.) Lindl (60 mesh) was purchased from Sichuan Tianzhi Co., Ltd. The extraction was performed as described in Zeng's study. In brief, *Anoectochilus roxburghii (Wall.) Lindl* powder was extracted with distilled water (1:50, w/v) at 70 ℃ for 1.5 hr. After suction, the aqueous extracts were concentrated to 1/10 of the initial volume with a rotary vacuum evaporator (RE-5210A, Shanghai Yarong Co., China) at 54℃. Then, 95% ethanol was added to the concentrated supernatant and this solution was kept at 4℃ for 24 hr, ARPs were collected by centrifugation (12000 rpm for 8 min, 20℃). The content of polysaccharide was measured by phenol-sulfuric acid method (sulfuric: phenol: polysaccharide = 5:1:2). Sulfuric acids (95%) were added and placed the samples in water bath at 100℃ for 10 min, absorbance was measured at 490 nm. Finally, the precipitates were washed with distilled water and freeze dried for further experiment. The ARPs components was identified by using High-performance liquid chromatography (HPLC, Agilent, Palo Alto, CA, USA), and separation was accomplished using a Shiseido C18 column (4.6 mm × 250 mm, 5 μm pore size). Molecular weight of

each component was determined by gel permeation chromatography (GPC) on the chromatograph Viscotek GPCmax VE2001 (Malvern Instruments, UK).

Animal and experimental design

6-week C57BL/6J mice were purchased from HFK Biological Technology Co. Ltd. Mice were housed in environmentally controlled animal rooms (23 ± 2 ℃, humidity 50%, range 30-70%) under a 12/12-hr day/night cycle and had access to food and water ad libitum. All mice were randomly divided into predefined groups. High-fat diet (HFD, D12492, 60 kcal% fat) supplemented with ARPs 1 mg/g and 3 mg/g (w/w) were used as low-dosage and high-dosage group separately, while HFD supplemented with Metformin (Meilunbio®, Dalian, China) 2 mg/g (w/w) was used as positive control group. Body weight, water intake and food intake were measured every week. Glucose tolerance test and insulin tolerance test were measured at 10-week, learning and memory ability were evaluated at 12-week. Mice at 14-week were euthanized with pentobarbital (100 mg/kg, i.p.), the hippocampus and colon were dissected rapidly. All experiments involving animals were approved by the Animal Ethics Committee of the Garment Hospital of Southwest Medical University.

Morris water maze (MWM) test

The water maze apparatus consisted of a round black pool (120 cm in diameter, 60 cm in height) containing Titanium oxide-mixed water (23-25 °C) and a platform (10 cm in diameter) submerged 1.0 cm under the water. The pool was divided into four quadrants equally named I, II, III, and IV, respectively. A black curtain was arranged around the pool to isolate interference from irrelevant visual cues, and four cues of assorted colours and shapes were fixed on the curtain opposite each quadrant as the only way for the mice to discriminate their position. During the training trial, mice were allowed to swim in the water while searching for the hidden platform for 90 s, and they were allowed to stay on it for 10 s upon finding the platform. Mice that failed to locate the platform in 90 s were guided to it, and then returned to their cage. The mice were trained twice per day at intervals of 60 min over five consecutive days. Twenty-four hours after the last training trial, the mice were tested for memory retention in a probe trial in the absence of the platform. The mice were allowed to swim in the pool for 90 s, and the time spent in the target quadrant where the platform had been fixed during the acquisition test was recorded. the time spent in the target quadrant, and the number of platform crossing times were monitored using a video camera (Sony, Tokyo, Japan) mounted over the maze and automatically recorded via a video tracking system.

Y maze test

The Y-maze apparatus consisted of three arms (30-cm long and 6-cm wide with 15-cm high walls) separated by an angle of 120°. The maze walls were constructed from dark black, polyvinyl plastic. The test consisted of two trials separated by an intertrial interval (2 hr). The first trial was 10 min in duration. Mice were placed in the start arm of the maze facing the terminal and allowed to explore two arms (the start arm and familiar arm) with the novel arm being blocked. In the second trial, mice were placed back in the maze in the same starting arm, with free access to all three arms for 5 min. Using a ceiling-mounted charge-coupled device camera, all trials were analyzed for the number of entries the mice made into each arm. The percentage of alternation (%) can be calculated using the following formula: [successive triplet sets (consecutive entries into three different arms)/total number of arm entries - 2] × 100%.

Glucose Tolerance Test (GTT) and Insulin Tolerance Test (ITT)

For GTT analysis, mice were injected intraperitoneally with glucose (1 g/kg) after an overnight fasting. Blood glucose levels were measured from the tail vein blood at 0, 15, 30, 60,90 and 120 min. For ITT analysis, after 4 hrs of fasting, mice were injected intraperitoneally with insulin (0.5 U/kg)，tail vein blood glucose levels of 0,15,30,60 90 and 120 min were detected.

Enzyme-linked immunosorbent assay (ELISA)

According to the manufacturer's instructions, commercial ELISA kit was used to determine the concentration level of serum LPS (Cloud-Clone Corp, Katy, TX, USA). and TNF- α (R&D systems, Minneapolis, USA).

Hematoxylin-eosin (H&E) staining

Hippocampus and colon samples were dissected and fixed in 4% paraformaldehyde for 24 hours, then the tissue was dehydrated and buried in paraffin. Tissue wax was cut into slices with a thickness of about 5 -30 μm (colon sections, 5 μm; brain serial sagittal sections, 30 μm). Each section was dewaxed, rehydrated and stained with hematoxylin and eosin. Tissue morphology was observed using a microscope (Leica, Milano, Italy) equipped with DFC350FX digital camera (Leica).

Immunohistochemistry

Sections were treated for 20 min with 0.6% H_2O_2 in PBS, permeabilised in PBS-Triton X-100 0.25% (PBS-Tx) for 30 min and blocked for 1 hr with 10% fetal bovine serum (FBS) in PBST×0.1%. TNFα (1:500

dilution, *Abcam*, ab34674) and ZO-1 (1:500 dilution, Abcam, ab59720) were incubated overnight at 4°C, followed by secondary antibodies (1:500 dilution) for 1 hr at room temperature. Positive signals were visualized using 3,3-diaminobenzidine (DAB) (Sigma-Aldrich, Saint Louis, MO, USA). Images were acquired with DM2500 microscope (Leica, Milano, Italy) equipped with DFC350FX digital camera (Leica).

Immunofluorescence

Sections were permeabilized in 1×PBS containing 0.25% Triton X-100 (PBS-TX) for 10 min, blocked with PBS-TX containing 10% FBS/1% bovine serum albumin/PBSTx 0.1% for 30 min at room temperature. After incubation with primary antibodies (anti-BDNF, ab108319, *Abcam*, 1:500) overnight, the sections were washed with PBS and incubated with the appropriate fluorescent secondary antibodies (1: 500, Alexa Fluor; Invitrogen) at room temperature for 1 hr. Confocal imaging was performed with a Leica TCS SP5 laser scanning confocal microscope (Leica, Milano, Italy).

16S rDNA sequencing

Following the manufacturer's instructions, QIAamp DNA fecal Mini Kit 206 was used to extract total DNA from mouse fecal samples. Amplification of V3-V4 region of bacterial 16s rRNA gene by PCR, and extracted from 2% agarose gel, then purified by VAHTS DNA Clean beads. QIIME (v1.8.0) was used to analyze the sequence data.

Quantitative real-time polymerase chain reaction analyses (RT-PCR)

Total mRNA was isolated from the mouse hippocampus or colon tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). 2 μg RNA was then reverse transcribed with the QuantiTect® Reverse Transcription Kit (Takara Ltd., Otsu, Japan) according to the manufacturer’s protocol. Quantitative Realtime PCR was performed using SYBR Green Realtime PCR Maser Mix (Bio-Rad, Hercules, CA, USA) to detect mRNA expression levels of target genes. The PCR reaction cycle program was as follows: 3 min of initial denaturation at 95 °C, then 40 cycles at 95 °C for 30 sec, 50 °C for 30 sec, 68 °C for 1 min 30 sec. All primer sequences are listed in Table S1.

Statistical analysis

Statistical Analysis and graphing were performed using GraphPad Prism 8.4.2. Data are presented as mean ± SEM, the significance of the differences between two groups was assayed by unpaired Student’s

t test. Multiple group comparisons were analyzed by one-way ANOVA followed with Bonferroni's *post hoc* test. $p < 0.05$ was considered statistically significant.

Results

ARPs ameliorated memory impairments triggered by HFD

The monosaccharide composition in ARPs was analyzed by HPLC. ARPs were heteropolysaccharides, the monosaccharide compositions supply important information for the polysaccharide structure, as shown in Supplementary Table 2. Five major monosaccharides including arabinose, galactaric acid, mannose, glucose and galactose were identified in polysaccharides, which further confirmed that all polysaccharides were heteropolysaccharides **(Fig. S1).**

Spatial learning and reference memory ability were often investigated using the MWM test[19]. As shown in Fig. 1a, compared with HFD, mice in ARPs group rapidly learned and memorized the location of the platform in the target quadrant, exhibited a significant decrease in the escape latency during the acquisition phase (**Fig. 1a**). Next, a probe trial was conducted by removing the platform. Mice in the ARPs -treated group spent a significantly more time swimming in the target quadrant than mice under HFD, and exhibited greater numbers of platform crossings (**Fig. 1b**), and these effects presented as a dose-dependent manner (**Fig. 1c-f**). Moreover, we evaluated the effect of ARPs on short-term spatial memory using the Y-maze test. HFD resulted in markedly decreased ratio of spontaneous alternation behavior compared with that of the chow diet mice, and these behaviors were partly recovered by administration of ARPs (**Fig. 1g-j**). Consistently, ARPs treatment also led to a significant increase in the number of arm entries compared with HFD group (**Fig. 1k-m**). The above results suggest that dietary ARPs supplementation effectively recovered the impairment of learning and memory in obese mice induced by HFD.

ARPs improved glucose and lipid metabolic disorders caused by HFD

In order to investigated whether ARPs can regulate glucose and lipids homeostasis, we next investigated the metabolic consequences of these mice. As shown in Fig. 2a, mice fed with a HFD showed increased body weight (**Fig. 2a**). Compared with mice in HFD group, the ARPs-treated mice exhibited markedly decreased body weight, serum glucose, triglyceride (TG) and total cholesterol (TC) levels (**Fig. 2b-d**). To observe the change in insulin sensitivity, we performed GTT and ITT analysis, mice on HFD showed higher insulin resistance and glucose intolerance compared with that in CD, while ARPs administration reversed these impairments (**Fig. 2e-h**). All these data suggest that ARPs effectively improved obesity, hyperglycemia and hyperlipidemia caused by HFD.

ARPs modulated regulators involved in cognitive function in hippocampus

Based on the observation that BDNF is essential in maintaining neural cells survival and promoting neurite outgrowth, synaptogenesis, memory and learning[20], while the microtubule-associated protein Tau is a key molecule in the pathogenesis of Alzheimer's disease[21]. We next performed experiments to determine whether ARPs modulate regulators involved in cognitive function. H&E staining results showed that HFD increased the number of nuclear pyknosis of hippocampal neurons, and ARPs treatment improved this phenomenon (**Fig. 3a**). Furthermore, we examined the expression of BDNF by immunofluorescence and observed an increase in hippocampus from ARPs-treated mice in comparison with mice on HFD (**Fig. 3b**), RT-PCR analysis of BDNF expression in hippocampus also reached the similar result (**Fig. 3c**). Meanwhile, ARPs administration significantly decreased the expression of Tau protein (the main component of amyloid plaques). Moreover, the mRNA expression of neurotrophin-3 (NT3), postsynaptic density protein-95 (PSD95, an important postsynaptic scaffolding protein) and fibroblast growth factor-21 (FGF21) in HFD mice hippocampus were downregulated, and dietary supplementation of ARPs recovered these effects **(Fig. 3d**). Taken together, these results suggest that ARPs promotes the survival of neuronal cells and improves cognitive impairment.

ARPs attenuated HFD-induced neuroinflammation in hippocampus

Inflammation is considered to be the most important pathophysiological mechanism of cognitive disorders, and Tumor necrosis factor-α (TNF-α) is reported to be closely associated with these impairments[22]. In this study, compared with CD group, mRNA expression of TNF-α, monocyte chemoattractant protein-1(MCP-1), interleukin- 6 (IL-6) and mouse EGF-like module-containing mucin-like hormone receptor-like 1 (F4/80) in hippocampus were increased in mice on HFD, while ARPs effectively abolished these effects **(Fig. 4a**). Meanwhile, the results of immunohistochemistry showed that ARPs improved the abnormal increased TNF- α in hippocampus induced by HFD **(Fig. 4b**). These data demonstrate that dietary supplementation of ARPs ameliorates hippocampal inflammation caused by HFD.

ARPs Attenuated HFD-induced Colon Inflammation

It is well documented that gut microorganisms and metabolites can stimulate inflammatory cytokines production and secretion, which triggers or exacerbates central inflammation and cognitive dysfunction[3]. In this study, RT-PCR analysis revealed that increased mRNA expression of TNF- α, MCP-1, IL-6 and F4/80 in colon were increased by HFD, and dietary supplementation of ARPs effectively reversed this trend **(Fig. 5a**). Meanwhile, immune-histochemistry results showed that supplementation of ARPs attenuated the abnormal increase of TNF-α in colon from mice on HFD **(Fig. 5b**). Our findings demonstrate that ARPs effectively attenuates HFD-induced inflammation of colon.

ARPs ameliorated impaired intestinal barrier induced by HFD

The integrity of intestinal epithelium is considered to be the first defense line of the gastrointestinal tract. Related studies have shown that long-term HFD can destroy the integrity of intestinal barrier and cause intestinal leakage[23]. In this study, H&E staining results demonstrated that the vacuoles of lipid accumulation in colonic villi from HFD mice were increased, PAS staining further showed that the number of mucus-secreting goblet cells, intestinal wall thickness and villus height were decreased by HFD, ARPs significantly improved these detrimental effects (**Fig. 6a**). By performing RT-PCR analysis, we found the expression of mucin-2 (Muc-2), the main components of intestinal mucus layer, a glycoprotein produced by Goblet cells, were down-regulated (**Fig. 6b**). Additionally, the expression of tight junction protein zonula occludens-1 (ZO-1) in colon from HFD mice was down-regulated (**Fig. 6c**). Moreover, mRNA levels of recombinant regenerating islet derived protein 3g (Reg3g) and Occludin-1 in colon also decreased by HFD, while ARPs reversed these effects (**Fig. 6b**). HFD has been reported to induce intestinal microbial disorders and enhance LPS production, which ultimately causing or aggravating systemic inflammation[24]. ELISA analysis further revealed that increased serum LPS and TNF-α levels induced by HFD were abolished by ARPs treatment partly (**Fig. 6d**). The above results suggest that dietary supplementation of ARPs can improve the integrity of intestinal barrier induced by HFD, and has a certain ability to regulate microbial metabolites.

ARPs reshaped the gut microbiome and ameliorated HFD-induced intestinal microbial disorders

In has been suggested that gut microbiota structure influence brain plasticity and cognitive function[25]. In this study, 16S rDNA sequencing analysis was used to detect whether ARPs could affect the intestinal microflora. Firstly, principal component analysis (PCA) showed significant differences in microflora structure among the three groups (**Fig. 7a**), and α-diversity and β-diversity of the microbiota from mice on HARPs were similar to mice on CD (**Fig. 7b**). In order to further study the specific changes of bacterial communities, we compared the relative abundance of dominant phyla and genera in the three groups, especially those that responded to ARPs. The results showed that HFD led to a significant increase in the abundance of eight bacterial genus including *Parabacteroides*, *Enterorhabdus*, *Lactoccus*, *Bacteroides*, *Lactocbacllus*, *Erysipelatoclostridium*, *Dubosiella* and *Faecalibaculum*, while ARPs supplementation significantly decreased the relative abundance of the above bacteria genus (**Fig. 7c**).

Discussion

Long-term high-fat diet has been shown to lead to central nervous system inflammation and damage of brain tissue, and eventually develop to neurodegenerative brain diseases such as Alzheimer's disease[26]. ARPs, one of the main components of *Anoectochilus roxburghii (Wall.) Lindl,* has been reported to

improve glucolipid metabolism and obesity-related complications[27]. Liu et al. found that ARPs gavage for 30 days decreased HFD-induced weight gain and hyperlipidemia[16]. Zeng et al. discovered that ARPs administration for 14 days possessed hepatoprotective effect against CCl4-induced hepatotoxicity in mice and the action might in part be through reducing oxidative stress, inflammation and apoptosis[28]. This study demonstrated that ARPs supplementation can effectively ameliorate cognitive dysfunction of HFD-induced obese mice. Mechanistically, these beneficial effects were associated with the alleviation of disorders of glucolipid metabolism, neuroinflammation and impaired intestinal function.

Cognitive ability and memory are influenced by neurotrophic factors including BDNF, which is primarily expressed in the central nervous system. Its biological effects include protection against neuronal injury and maintenance of neuronal survival and physiological function in the mature central and peripheral nervous systems[20]. Wang et al. reported that BDNF levels were significantly reduced in hippocampus of HFD-induced obese mice, which was associated with the induction of cognitive impairment[29]. Additionally, accumulation of hyperphosphorylated tau proteins positively correlates with cognitive dysfunction in Alzheimer's disease. Reduction of Tau protein prevented neuronal loss and reversed pathological deposition [30]. In this study, dietary supplementation with ARPs increased the expression of BDNF and decreased Tau protein expression in the hippocampus of obese mice, which may account for the improved cognitive function.

As previous studies pointed, ARPs is a heteropolysaccharide with an average molecular weight of about 22235~66004 Da, mainly composed of arabinose, mannose, glucose and galactose, most of which have high molecular weight and polarity[31]. However, the blood-brain barrier (BBB) restricts the passage of molecules larger than 500Da[32], Thus, we speculated that ARPs is hard to pass through the BBB and act directly on the central nervous system. It is well documented that HFD can promote inflammation and insulin resistance in the whole body including the brain, thus damaging hippocampal neurons and synapses and causing cognitive dysfunction[33]. Moreover, HFD induces gut microbiota disorders and increases the LPS production, which further aggravated the inflammation and the consequent cognitive dysfunction[34]. Our research has revealed the positive role of ARPs in improving glycolipid metabolism, insulin resistance and inflammation. Meanwhile, ARPs can improve the impaired intestinal barrier and microbiota disorders, accompanied by decreased LPS and TNF-α levels. Therefore, we conclude that ARPs may improve the cognitive impairment induced by obesity via the “gut-brain” axis.

Previous studies have shown that the gut microbiota can influence brain plasticity and cognitive function[4, 35]. Huang et al. found that *Bacteroides paracasei* can modulate age-related cognitive decline by altering communication in the gut-brain axis[36]. In this study, 16S rDNA sequencing analysis confirmed that dietary supplementation with ARPs reduced the relative abundance of 8 certain taxa.

Lactobacillus, *Faecalibaculum* and *Parabacteroides* have been considered as potential beneficial bacteria in some research[37]. However, these species display some degree of pathogenicity under particular conditions. A recent study found that cognitive impairment can affect the relative abundance of *Parabacteroides*, which has been regarded as a risk indicator of mild cognitive impairment[38]. Considering that the relationship between gut microbiota and cognitive function is complicated, more studies should be carried out to understand the underlying mechanism of ARPs on cognitive dysfunction induced by HFD，but the period is relatively long, we can only demonstrate the related outcomes in future studies.

Conclusion

In summary, ARPs ameliorated memory capacity and cognitive impairment in obese mice by improving neuroinflammation induced by HFD. Meanwhile, ARPs supplementation restored intestinal barrier integrity and balanced gut microbial dysbiosis. The results confirmed the potential effect of ARPs on ameliorating cognitive impairment and providing a theoretical basis for the development of functional herbal extracts.

Abbreviations

ARPs, *Anoectochilus roxburghii (Wall.) Lindl* polysaccharides; HFD, high-fat diet; CD, chow diet; HARPs, HFD supplemented with *Anoectochilus roxburghii (Wall.) Lindl* polysaccharides; MWM, Morris water maze; GTT, glucose tolerance test; ITT, insulin tolerance test; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin-eosin; RT-PCR, quantitative real-time polymerase chain reaction analyses; TG, triglyceride; TC, total cholesterol; PAS, periodic acid-Schiff; PCA, principal component analysis; LPS, lipopolysaccharide; MUC-2, mucoprotein-2; Reg3g, recombinant regenerating islet derived protein 3g; ZO-1, zonula occludens-1; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin- 6; BDNF, brain-derived neurotrophic factor; NQO1, quinone oxidoreductase-1; NT3, neurotrophin-3; PSD95, postsynaptic density protein-95; F4/80, mouse EGF-like module-containing mucin-like hormone receptor-like 1; FGF21, fibroblast growth factor-21; BBB, blood-brain barrier

Declarations

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. We have uploaded the 16S rDNA raw data on the NCBI database, and the accession number is PRJNA754503. The link is as follows: https://submit.ncbi.nlm.nih.gov/subs/

Ethics approval and consent to participate

All the experimental protocols were approved by the Animal Experimental Ethical Committee, the Affiliated Hospital of Southwest Medical University (Ref No.20210223-078). All participants provided written informed consent.

Authors' contributions

Y.X. and H.Y established the study. F.L. and Z.W. designed the experiments and contributed equally to this work. T.D. and W.Q. performed the experiments, analyzed the data and edited the manuscript. Y.Y. and Z.C. contributed to literature searches, article selections and RT-PCR analysis. Q.D. and Q.W. performed the Morris water maze. T.Y. performed the Y Maze Test. All authors have read and approved the final manuscript.

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Consent for publication

Not applicable.

Conflicts of interest

The authors declare no competing interest.

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Figures

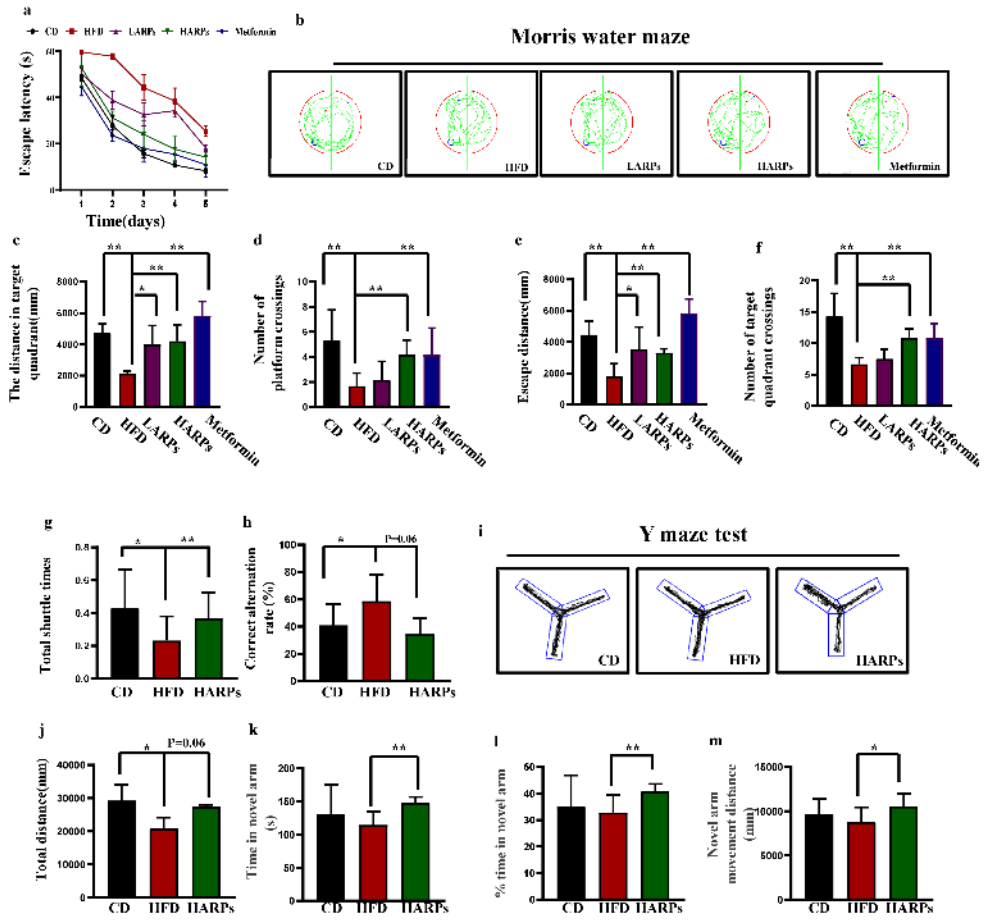


Figure 1

APPs ameliorated memory impairments triggered by HFD (a) The latency to find the hidden platform was measured during training trials (n=6). (b-h) During probe trials, Morris water maze(b), the distance in target quadrant of mice in the MWM tests (c), number of platform crossings (d), escape distance (e), number of target quadrant crossings (f), total shuttle times (g), and correct alternation rate were recorded (h) (n=6). (i) Y-maze test (j) Total distance (mm) (k)Time in novel arm (s) (l)%time in novel arm (m) Novel

arm movement distance (mm) (n=6). Data are presented as mean ± SEM, *p< 0.05, **p< 0.01, HARPs compared with HFD. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides. HFD, high-fat diet; HARPs, HFD + ARPs.

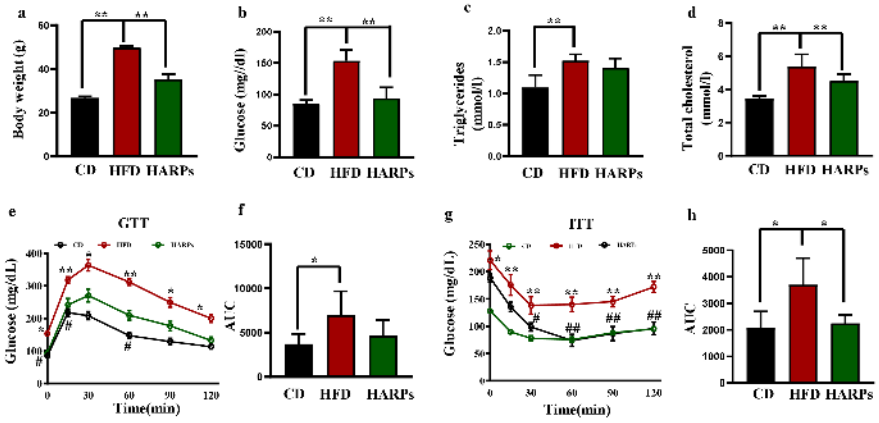


Figure 2

ARPs improved glucose and lipid metabolic disorders caused by HFD (a) Body weight of the three groups (n=6). (b-d) The levels of serum glucose (b), triglyceride (c) and total cholesterol (d) were detected (n=6).

(e-f) GTT of the four groups and the associated area under the curve (AUC) values (n=6). (g-h) ITT of the four groups and the associated area under the curve (AUC) values (n=6). Data are presented as mean ± SEM, *p < 0.05, **p < 0.01, HARPs compared with HFD. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides; GTT, glucose tolerance test; ITT, insulin tolerance test; HFD, high-fat diet; HARPs, HFD + ARPs; AUC, area under the curve.

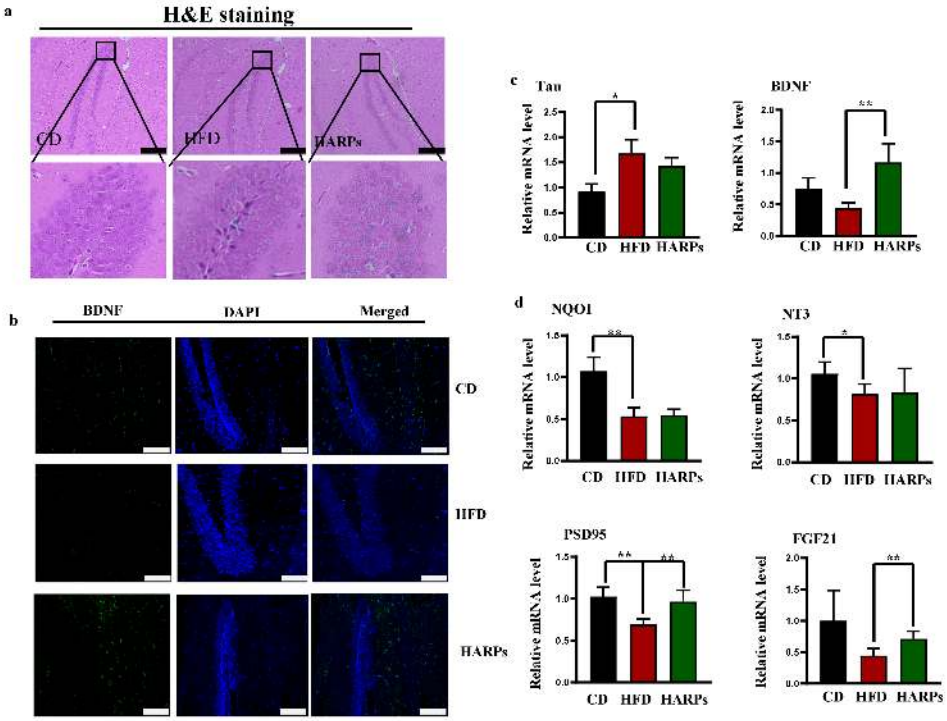


Figure 3

ARPs modulated regulators involved in cognitive function in hippocampus (a) H&E staining of hippocampus. Scale bar = 100μm.(b) Immunofluorescence staining of BDNF in hippocampus. (c)The mRNA levels of Tau and BDNF in mice hippocampus (n=6). Scale bar = 50 μm. (d) the mRNA levels of NQO1, NT3, PSD95 and FGF21 in mice hippocampus (n=6). Data are presented as mean ± SEM, *p< 0.05, **p< 0.01. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides; HFD, high-fat diet; HARPs, HFD + ARPs.

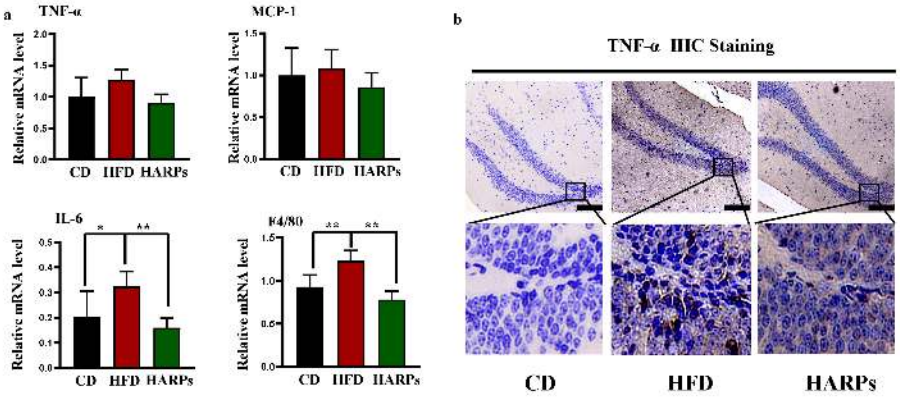


Figure 4

ARPs attenuated HFD-induced neuroinflammation in hippocampus (a)the mRNA levels of TNF-α, MCP-1, IL-6 and F4/80 in mice hippocampus (n=6). (b) Representative images of TNF-α immunohistochemical staining in mice hippocampus. Scale bar = 100 μm. Data are presented as mean ± SEM, *p< 0.05, **p< 0.01. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides; HFD, high-fat diet; HARPs, HFD + ARPs.

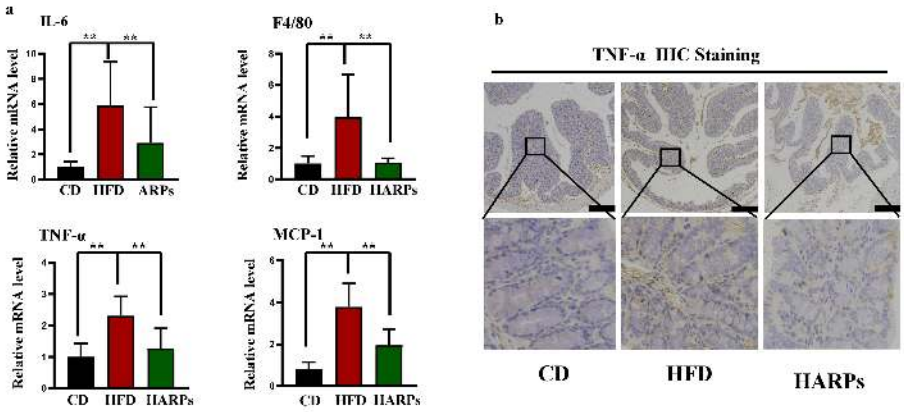


Figure 5

ARPs attenuated HFD-induced colon inflammation (a) the mRNA levels of TNF-α, MCP-1, IL-6 and F4/80 in mice colon (n=6). (b) Representative images of TNF-α immunohistochemical staining in mice colon. Scale bar = 50 μm. Data are presented as mean ± SEM, *p< 0.05, **p< 0.01. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides; HFD, high-fat diet; HARPs, HFD + ARPs.

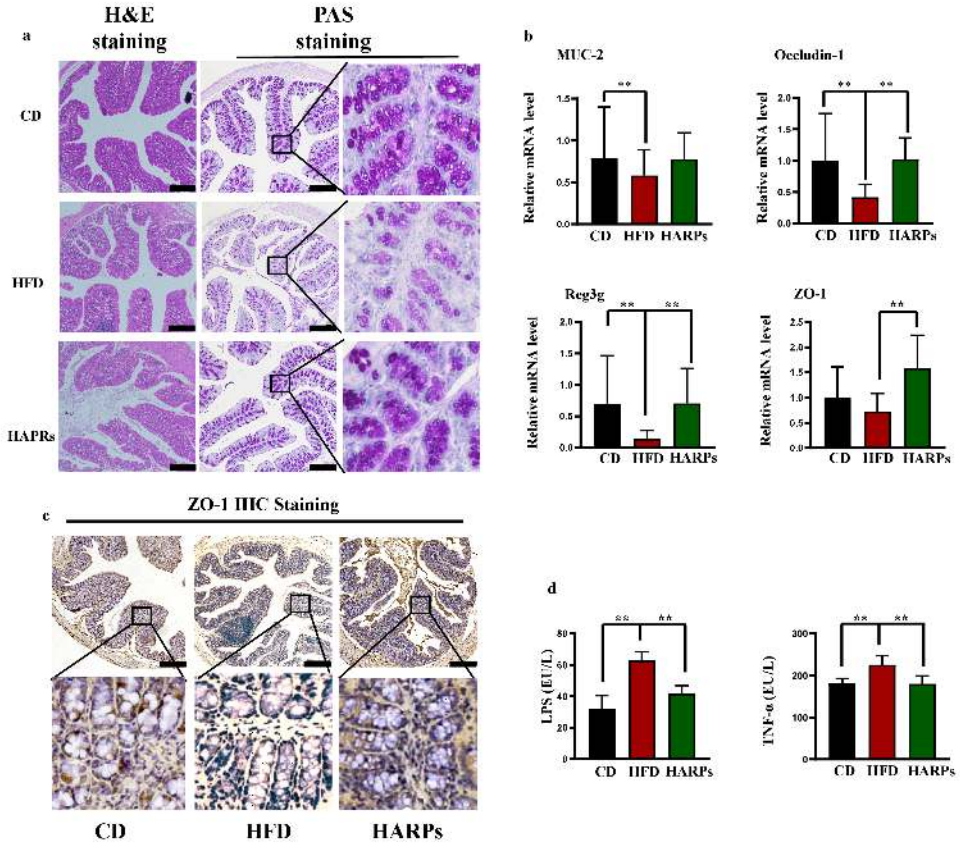


Figure 6

ARPs ameliorated intestinal barrier induced by HFD (a) H&E staining and PAS staining of colon. Scale bar = 50 μm. (b) the mRNA levels of Muc-2, Occludin-1, Reg3g and ZO-1 in colon (n=6). (c) Representative images of ZO-1 immunohistochemical staining in mice colon. Scale bar = 50 μm. (d) ELISA analysis of serum LPS and TNF-α levels (n=6). Data are presented as mean ± SEM, *p< 0.05, **p< 0.01. H&E, hematoxylin-eosin; PAS, Periodic Acid-Schiff; ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides; HFD, high-fat diet; HARPs, HFD + ARPs.

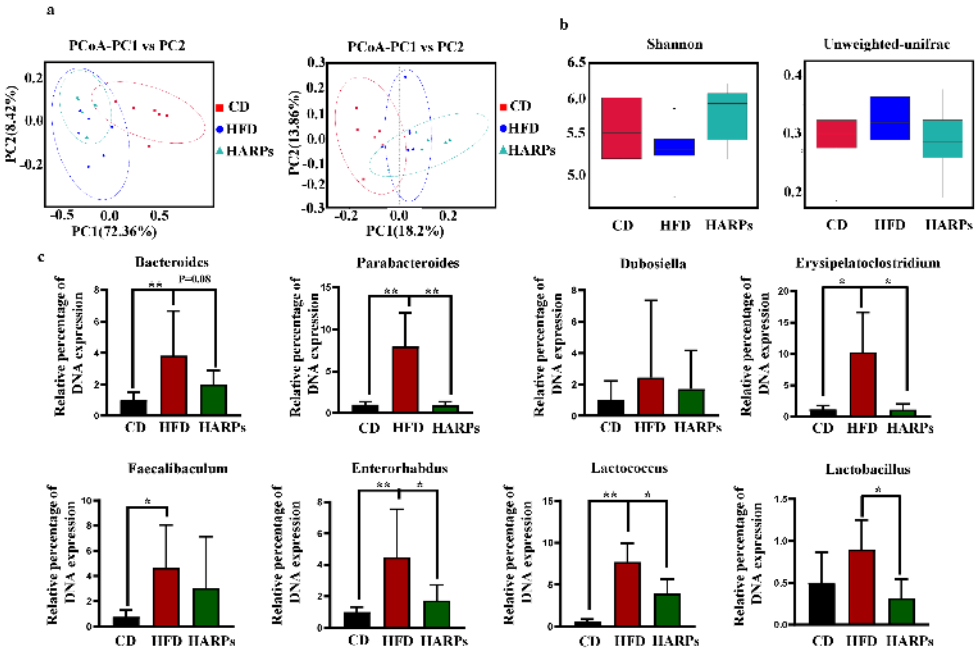


Figure 7

ARPs reshapes the gut microbiome and ameliorates HFD-induced intestinal microbial disturbance (a) PCoA show the beta diversity of microbial communities (n=6). (b) The unweighted-unifrac and Shannon analysis on OTUs (n=6). (c) The relative abundance of Parabacteroides, Enterorhabdus, Lactoccus, Bacteroides, Lactocbacllus, Erysipelatoclostridium, Dubosiella, Faecalibaculum (n=5). Data are presented as mean ± SEM, *p< 0.05, **p< 0.01. PCoA, principal component analysis; OTUs, operational taxonomic units. ARPs, Anoectochilus roxburghii (Wall.) Lindl Polysaccharides. HFD, high-fat diet; HARPs, HFD + ARPs.

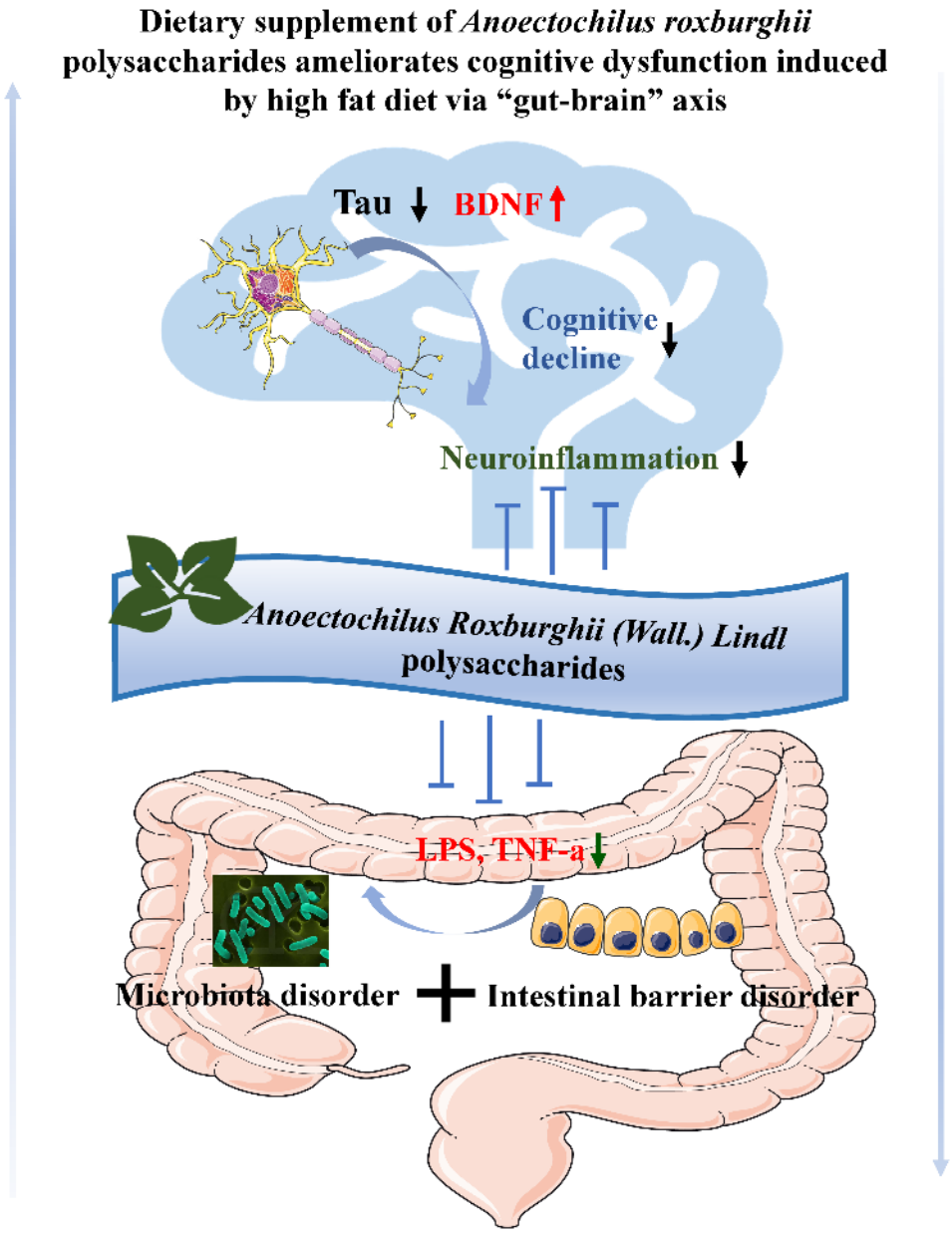


Figure 8

Graphic abstract. ARPs supplementation restored HFD-induced glucose and lipid metabolic disorders, reshaped the gut microbiome and ameliorated impaired intestinal barrier. All these effects were possibly associated with the improvement of neuroinflammation, insulin resistance, and cognitive dysfunction.

Supplementary Files

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- 20210829SupplementaryMaterials.docx