Dietary Supplement of *Anoectochilus Roxburghii* Polysaccharides Ameliorates Cognitive Dysfunction Induced by High Fat Diet via “Gut-Brain” Axis

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Research Article

Keywords: Anoectochilus roxburghii polysaccharides, “gut-brain” axis, cognitive dysfunction, gut microbiota, diet-induced-obesity

Posted Date: August 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-732344/v1

Abstract

Background *Anoectochilus roxburghii*, a traditional Chinese herbal medicine, has been widely used for treating numerous metabolic diseases. *Anoectochilus roxburghii* polysaccharide (ARPs) is an important constituent of *Anoectochilus roxburghii*. This study tried to investigate whether ARPs could improve cognitive dysfunction in diet-induced obesity.

Methods: C57BL/6J male mice were randomly divided into the normal chow diet group (CD), the high-fat diet group (HFD) and HFD supplemented with ARPs (HARPs). Morris water maze test and Y maze test were used to evaluate spatial learning and memory. Serum TNF-α and LPS concentrations were detected by ELISA. Genes involved in Neurotrophic factors, inflammation and Intestinal permeability were detected by RT-PCR. Immunohistochemistry was used to detect TNF-α and ZO-1 proteins. BDNF in hippocampus was detected by immunofluorescence staining. 16S rDNA sequencing was used to detect intestinal microbial diversity.

Results: Dietary supplement of ARPs ameliorates cognitive dysfunction induced by HFD. Compared with HFD mice, body weight, plasma glucose, total cholesterol and triglyceride levels in HARPs group were decreased significantly. Moreover, the cognitive function and memory of HFD mice were decreased, while ARPs abolished these effects. In addition, Tau protein levels were decreased and BDNF increased significantly in hippocampus from HARPs mice. Furthermore, compared with HFD group, IL-6 and TNF-α levels in hippocampus and colon from HARPs mice were decreased. Moreover, ARPs increased the tight junction protein levels and intestinal microbiota diversity significantly.

Conclusions: Diet supplemented with ARPs prevented mice from HFD-induced cognitive dysfunction, indicated that ARPs has a good effect on obesity-related neuropathy. Mechanically, these effects at least in part attribute to the “gut-brain” axis regulation, and which can provide reference for the development of ARPs as functional foods in the future.

Introduction

Cognitive dysfunction is a syndrome manifested primarily by impairment of learning and memory functions [1]. A great deal of research suggests that obese individuals are at high risk of neurodegenerative disorders, and that obesity and over-weight are associated with deficits in learning, memory, executive function as well as potential brain atrophy [2, 3]. Given the close correlation between obesity and cognitive impairment, ameliorating obesity may have a positive impact on the cognitive dysfunction.

Inflammation is thought to be involved in the pathophysiology of cognitive impairment and dementia, which is associated with the neuropathological features of Alzheimer’s disease [4, 5]. Clinical studies including obese subjects have shown that systemic inflammation is positively associated with symptoms of cognitive decline [6]. Neuroinflammation causes central insulin resistance, and ultimately leading to hyperphosphorylation of Tau protein and induce apoptosis of neuronal cells, which lead to

neurodegenerative diseases [3]. Park et al. showed that diet-induced-obesity leads to reduced brain-derived neurotrophic factor (BDNF) secretion in hippocampus and cerebral cortex that affect cognitive function [7]. In addition, many inflammatory and metabolic diseases are closely related to intestinal dysfunction[8]. Obesity causes changes in the composition of the intestinal microflora and metabolites, and long-term high-fat diet leads to extensive accumulation of lipids in intestinal cells, trigger intestinal oxidative stress, and damage the intestinal barrier [9]. In obese individuals, large quantities of lipopolysaccharides (LPS) in intestinal enter the blood circulation, which stimulates inflammatory factors secretion that triggers or intensifies Neuroinflammation and even cognitive dysfunction [10–12].

It has been shown that some herbal prescriptions and monomers such as *ginkgo biloba* preparations and *rhodiol* glycosides can improve cognitive dysfunction [13, 14]. *Anoectochilus roxburghii* is classified as a genus of open-lipped orchids. *Anoectochilus roxburghii* polysaccharides (ARPs) is one of the main components of goldenseal, which has antioxidant, anti-inflammatory, hypolipidemic, hypoglycemic, antibacterial, and antitumor pharmacological activities [15–18]. 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 [19]. Moreover, Wang et al. showed that *Anoectochilus roxburghii* extract significantly delayed aging in D-galactose-induced dementia model [20]. Another study showed that ARPs lowered the pH in the cecum and increased its content of probiotic *bifidobacterial* [21]. 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

Anoectochilus roxburghii

Anoectochilus roxburghii (60 mesh) was purchased from Sichuan Tianzhi Co., Ltd. The extraction was performed similarly as reported in Zeng's study [18].

Animal and experimental design

C57BL/6J mice (6-week-old), body weight (20-22g), was purchased from HFK Biological Technology Co. Ltd. Mice were housed in environmentally controlled animal rooms (temperature 20 ± 2℃, humidity 50%, range 30-70%) under a 12/12-h day/night cycle and had access to food and water *ad libitum*. All experiments involving animals were approved by the Animal Ethics Committee of the Garment Hospital of Southwest Medical University. All mice were randomly divided into three groups: chow diet (CD), high-fat diet (HFD) and HFD supplemented with *Anoectochilus roxburghii* polysaccharides 200mg/kg/day (HARPs). 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 deeply anesthetized with hexane. The hippocampal and colon were separated quickly.

Morris water maze (MWM) test

MWM device consists of a round black pool (straight diameter 150cm, high 35cm) and a video analysis system. The pool was evenly divided into four quadrants, and the pool filled with water (23-25℃) mixed with nontoxic white paint (titanium oxide). Mice were trained to mount submerged escape platform (15cm diameter) in a restricted region of the pool. During the 5 consecutive days, mice were placed into the water facing the wall and were allowed to swim for 60 seconds. The trial ended when a mouse climbed on to the platform or after 60 seconds. If a mouse did not locate the platform during the trial, it was placed on the platform by the experimenter and stay for 30 seconds. After four days, a memory test was performed in absence of tIhe platform. Mice were released from the four start points to the platform location and allowed to swim freely in the pool for 60s, and the escape latency and swimming paths were recorded.

Y maze test

Mice were placed in Y-maze and allowed to explore freely for 6 min. The total number of arm entry and the correct spontaneous alternation of arm entry were recorded. The correct rate of spontaneous alternation of arm entry = (correct number of spontaneous alternations / (total number of arms-2)) × 100%. New different arm exploration experiment: Three arms of Y-maze were defined as the starting arm, new arm, and other arms. During the training period, new arm was blocked, mice were put into the starting arm, and moved freely for 10 min. After training, mice returned to the cage. After 60 min, the testing period was carried out. During testing period, new arm baffle was removed, the mice were placed in the starting arm, and moved freely in three arms for 5 min. The residence time, times and distance of each mouse in each arm were recorded.

Assays of glucose tolerance test (GTT) and insulin tolerance test (ITT)

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

Enzyme-linked immunosorbent assay (ELISA)

According to the manufacturer's suggestion, commercial ELISA kit was used to determine the concentration level of serum LPS and TNF- α. The serum was centrifuged at 12 000rpm for 5 min, and the supernatant was taken as ELISA sample.

Histological analysis

For hematoxylin-eosin (H&E) staining, when the mice deeply anesthetized, the hemisphere was immediately removed and fixed in 4% paraformaldehyde / PBS fixed solution for 24 hours, then the tissue was dehydrated and buried in paraffin. The wax was cut into 5-μm slices, dewaxed and stained with

hematoxylin-eosin, and the tissue morphology was observed under light microscope. For immunohistochemistry, tissue sections were dewaxed and sealed, the first antibody was incubated overnight at 4 ℃, the second antibody was incubated for 60 minutes, horseradish enzyme labeled streptomycin working solution incubated for 10 minutes, hematoxylin re-staining for 3 minutes, washing with running water for 15 minutes, 5% glacial acetic acid differentiation for 30s, gradient ethanol dehydration, clarification in xylene, resin seal and observed under light microscope. For immunofluorescence, the operation steps similar to immunohistochemistry. After the corresponding first antibody was incubated for the tissue section overnight, the section was removed the next day, recovered at room temperature for 15 minutes, and the corresponding fluorescent second antibody was added for 2 hours. Sealed with anti-fluorescence attenuation film containing DAPI, observed and photographed under fluorescence microscope.

16S rDNA sequencing

Following the manufacturer's recommendation, 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 RNA from mouse tissues was prepared with Trizol reagent (Life Technologies) according to the manufacturer’s instructions. 2 μg RNA was the reverse transcribed with the QuantiTect® Reverse Transcription Kit (Qiagen GmbH) according to the manufacturer’s protocol, then using Power Up SYBR Green Master Mix (Thermo Scientific) on a CFX96 C1000 Thermal Cycler (Bio Rad). Differences in expression were evaluated by the comparative cycle threshold method using 18s as control. The primer sequences used for the RT-PCR experiments are listed in Table 1.

Table 1 Primer Sequence

Primer	Forward Primer	Reverse Primer
MCP-1	TCTGTGCCTGCTGCTCATAG	GCTTGTCCAGGTGGTCCATG
F4/80	ATGTCTGGCTTGCACCTAGTA	CCCCAAAGCGAGTAACAAATTCT
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
IL-6	TTCCATCCAGTTGCCTTCTTG	TATCCTCTGTGAAGTCTCCTCTC
BDNF	CTCCGCCATGCAATTTCCACT	GCCTTCATGCAACCGAAGTA
Tau	AACCAGATCGGGATGGAGTTC	CAGCGACCGAGGGATGTACT
ZO-1	TGGGAACAGCACACAGTGAC	GCTGGCCCTCCTTTTAACAC
Occludin-1	AGTACATGGCTGCTGATG	CCCACCATCCTCTTGATGTGT
Reg3g	CCTCAGGACATCTTGTGTCTGTGCTC	TCCACCTCTGTTGGGTTCATAGCC
MUC-2	CACTGCGATGCCAACGACA	GCCACTAACTGCTTGTTCACCTGTA
18s	TTGACGGAAGGGCACCACCAG	GCACCACCACCCACGGAATCG

Statistical analysis

All values in the figures and tables are presented as mean ± SEM. Data were analyzed using ANOVA followed by post hoc LSD test. Statistical Analysis and graphing were performed using GraphPadPrism8.4.2. P value < 0.05 was considered as statistically significant.

Results

ARPs improved glucose and lipid metabolism disorders caused by HFD

In order to study investigated whether ARPs can regulate body weight, glucose and lipids homeostasis, we measured the following indicators. As illustrated in Fig. 1a, mice fed with a HFD were visibly heavier than the CD and HARPs. Compared with HFD, the mice in HARPs markedly decreased the serum glucose, triglyceride (TG) and total cholesterol (TC) levels (Fig. 1b-d). At week 12, the mice in HFD emerged insulin resistance accompanied by glucose intolerance compared with that in CD, while ARPs reversed these changes (Fig. 1e-h). These results suggest that ARPs effectively improved obesity and abnormal levels of serum lipids and blood glucose caused by HFD.

ARPs ameliorated memory impairments triggered by HFD

Spatial learning is often assessed using maze tasks such as the MWM or Y maze test [22, 23]. In this study, we use the MWM to assess the learning and memory ability. During the period of the MWM test, mice in HARPs group produced a decrease in the escape latency when compared with HFD (Fig. 2a). Simultaneously, during the probe trial (Fig. 2b), HARPs mice exhibited a significant increase in the

average time spent in the target quadrant and exhibited greater numbers of platform crossings than HFD mice, which indicates that ARPs could improve HFD-induced memory impairments (Fig. 2c-f). Consistently, Y maze test also indicated that ARPs ameliorated memory impairments triggered by HFD significantly (Fig. 2c-f). The above results suggest that dietary ARPs supplementation effectively inhibited the impairment of learning and memory in obese mice induced by HFD.

ARPs improved cognitive disorder induced by HFD

BDNF is essential in maintaining neural cells survival and promoting neurite outgrowth, synaptogenesis, memory and learning [24]. In addition, microtubule-associated protein Tau is a key molecule in the pathogenesis of Alzheimer's disease [3]. In this study, the morphology of hippocampal neurons was observed by H&E staining. The results showed that 12-week HFD increased the number of nuclear pyknosis of hippocampal neurons, and ARPs could improve this phenomenon (Fig.3a). Furthermore, immunofluorescence and RT-PCR analysis were used to analyze the content of neurotrophic factors in hippocampal. The immunofluorescence results showed that 12-week HFD decreased BDNF levels in hippocampal, and ARPs effectively reversed this trend (Fig.3b). RT-PCR analysis of BDNF expression in hippocampal also reached the similar result, meanwhile, Tau protein expression were decreased by ARPs treatment. Moreover, the mRNA expression of quinone oxidoreductase-1 (NQO1), neurotrophin-3 (NT3), postsynaptic density protein-95 (PSD95) and recombinant human fibroblast growth factor-21 (FGF21) in mice hippocampus were decreased in HFD mice, and dietary supplementation of ARPs reversed the trend (Fig.3c-d). Taken together, these results suggest that ARPs promotes the survival of nerve cells and improve cognitive impairment.

ARPs attenuated HFD-induced neuroinflammation in hippocampal

Inflammation is thought to be the most important pathophysiological mechanism of cognitive disorders [4], and Tumor necrosis factor-α (TNF-α) is reported to be closely associated with these impairments[25]. In this study, the results of immunohistochemistry showed that ARPs improved the abnormal increased TNF- α induced by HFD. RT-PCR results revealed that compared with CD group, the 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 hippocampal were increased in mice on HFD, while ARPs effectively abolished these effects. These data demonstrate that dietary supplementation of ARPs ameliorates hippocampal inflammation caused by HFD.

ARPs attenuated HFD-induced colon inflammation

Gut microorganisms and metabolites can stimulate inflammatory factors production and secretion, which triggers or exacerbates central inflammation and even induces cognitive dysfunction [5]. In this study, immunohistochemistry results showed that supplementation of ARPs attenuated the increased TNF- α expression in colon induced by HFD. Meanwhile, 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.

ARPs ameliorated intestinal barrier and microbial metabolite disorder induced by HFD

The integrity of intestinal epithelium is considered to be the first defense line of the gastrointestinal tract [26]. Studies have shown that HFD lead to damaged intestinal epithelial barrier by increasing inflammation [27]. In this study, H&E staining results demonstrated that the vacuoles of lipid accumulation in colonic villi from HFD mice were increased, and ARPs significantly improved these phenomenons (Fig.6a). Moreover, PAS staining showed that the number of mucus-secreting goblet cells, intestinal wall thickness and villus height were decreased by HFD (Fig.6a), and RT-PCR analysis further revealed the expression of MUC-2 were down-regulated (Fig.6b). By contrast, dietary supplementation of ARPs reduced the damage of epithelial integrity caused by HFD and increased the number of goblet cells.

Related studies have shown that long-term HFD can destroy the integrity of intestinal barrier and cause intestinal leakage [8]. As shown in (Fig. 6c), the expression of tight junction protein zonula occludens-1 (ZO-1) in colon from HFD mice was down-regulated. Moreover, mRNA levels of recombinant regenerating islet derived protein 3g (Reg3g), mucoprotein-2 (MUC-2) and Occludin-1 in colon (Fig. 6b) also reached the same result. while ARPs reversed these effects.

HFD has been reported to induce intestinal microbial disorder and increase LPS production, which lead to increased LPS and inflammatory factors levels in the circulatory system, causing or aggravating systemic inflammation [27]. In this study, the contents of serum LPS and TNF- α in mice on HFD were significantly increased, and ARPs could effectively ameliorate the phenomenon (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 intestinal microbial metabolites.

ARPs ameliorated HFD-induced intestinal microbial disturbance

In has been suggested that gut microbiota influence brain plasticity and cognitive function [28]. In this study, 16S rDNA sequencing analysis was used to detect whether ARPs could affect the composition of 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

It is well known that a long-term high-fat diet leads to obesity, hypertension, and type 2 diabetes, other metabolic disorders. It also leads to central nervous system inflammation and damage to brain tissue,

eventually developing to neurodegenerative brain diseases such as Alzheimer's disease [29–31]. ARPs, one of the main components of *Anoectochilus roxburghii*, has been widely reported to improve glucolipid metabolism and obesity-related complications. Liu *et al.* found that ARPs gavage for 30 days decreased HFD-induced weight gain and hyperlipidemia [19]. Zeng et al. discovered that ARPs infusion for 14 days effectively lowered blood glucose in a CCl_4-induced mouse diabetes model [32]. This study evaluated the effects of ARPs on learning and memory in HFD-induced obese mice, and found ARPs significantly improved HFD-induced weight gain and disorders of glucolipid metabolism. All these effects were possibly associated with improvement of inflammation, insulin resistance, neuronal damage, and intestinal dysfunction.

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 [24]. Wang *et al.* reported that BDNF levels were significantly reduced in hippocampal from HFD-induced obese mice and which was associated with the induction of cognitive impairment [24]. Tau protein is considered to be a key molecule in the pathogenesis of Alzheimer’s disease. Hyperphosphorylation of Tau protein leads to synaptic damage and neurodegeneration [3]. In this study, dietary supplementation with ARPs increased the expression of BDNF and decreased Tau protein expression in the hippocampal tissue of obese mice, which may account for the improved cognitive function.

Inflammation and insulin resistance (IR) have been reported to be associated with the pathophysiology of cognitive impairment[2, 6]. Obesity-related inflammation in peripheral tissues and the presence of proinflammatory factors in the circulation eventually involve the brain, thus triggering cognitive impairment[5, 10]. Guo *et al.* found that ARPs had anti-inflammatory effects in rats with type II collagen-induced arthritis, including reducing TNF-α, IL-1β, and IL-6 levels, and promotion of the release of IL-10 [33]. Consistently, our study also found ARPs improved inflammation and IR induced by HFD.

It has been shown that HFD cause inflammation and damage the intestinal epithelial barrier [34]. In this study, the expression of tight-junction proteins in the colon epithelium was reduced in mice on HFD, ARPs supplementation protected against this effect. Impaired intestinal barrier integrity impelled LPS and intestinal inflammatory factors to enter the circulation, and trigger or exacerbate systemic inflammation [26, 27]. In this study, increased LPS and TNF-α concentrations induced by HFD was abolished by ARPs. In addition, Dietary supplementation with ARPs increased the number of goblet cells, which provided a rich source of nutrients for the growth of intestinal microorganisms [27].

Previous studies have found that the gut microbiota can influence brain plasticity and cognitive function [35, 36]. Huang *et al.* reported that probiotic supplements are potential novel agents for the treatment of age-related diseases [28]. They found that *Bacteroides paracasei* can modulate age-related cognitive decline by altering communication in the gut-brain axis [27]. 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 [37]. In this study, 16S rDNA sequencing analysis confirmed that dietary supplementation with ARPs improved HFD-induced gut microflora dysbiosis.

Conclusion

In summary, ARPs ameliorated memory capacity and cognitive impairment in obese mice by increasing the expression of BDNF and improving neuroinflammation. Meanwhile, ARPs supplementation restored intestinal barrier integrity and decreased intestinal and restored HFD-induced gut microbial dysbiosis. The results confirmed the potential of ARPs as probiotics to ameliorate cognitive impairment and provided a theoretical basis for the development of functional herbal extracts. The mechanisms of ARPs on cognitive function and gut microbiota remained to be determined.

Abbreviations

ARPs, *Anoectochilus roxburghii* polysaccharides; HFD, high-fat diet; CD, chow diet; HARPs, HFD supplemented with *Anoectochilus roxburghii* 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; SOD, superoxide dismutase; MDA, malonaldehyde; 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.

Declarations

Acknowledgement

All authors acknowledge the support received from the Laboratory of Traditional Chinese Medicine and Laboratory of Pharmacological, Southwest Medical University. Authors would also like to thank International Science Editing Co., Ltd, China for editorial support on this manuscript.

Authors' contributions

YXP and HYL established the study and obtained the funding. FLY and ZWL designed the experiments. TDM, WQM., LYZ, and ZXY, performed the experiments, analyzed the data and edited the manuscript. XCJ, LMY and ZCB. contributed to literature searches, article selections and RT-PCR analysis. QDL, PMX and QWQ performed the Morris water maze and Y maze test. ZJY and WF prepared the polysaccharides. All authors have read and approved the final manuscript.

Funding

This study was supported by research funding from the Science and Technology Planning Project of Sichuan Province (No.2019YFS0180), Doctoral Research Initiation Fund of Affiliated Hospital of Southwest Medical University (No.21032), the Foundation for Young Scholars of Southwest Medical University (No.2020ZRQNB030, No.2019-ZQN-125, No.2019-ZQN-151).

Availability of data and material

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

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.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare no competing interest.

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Figures

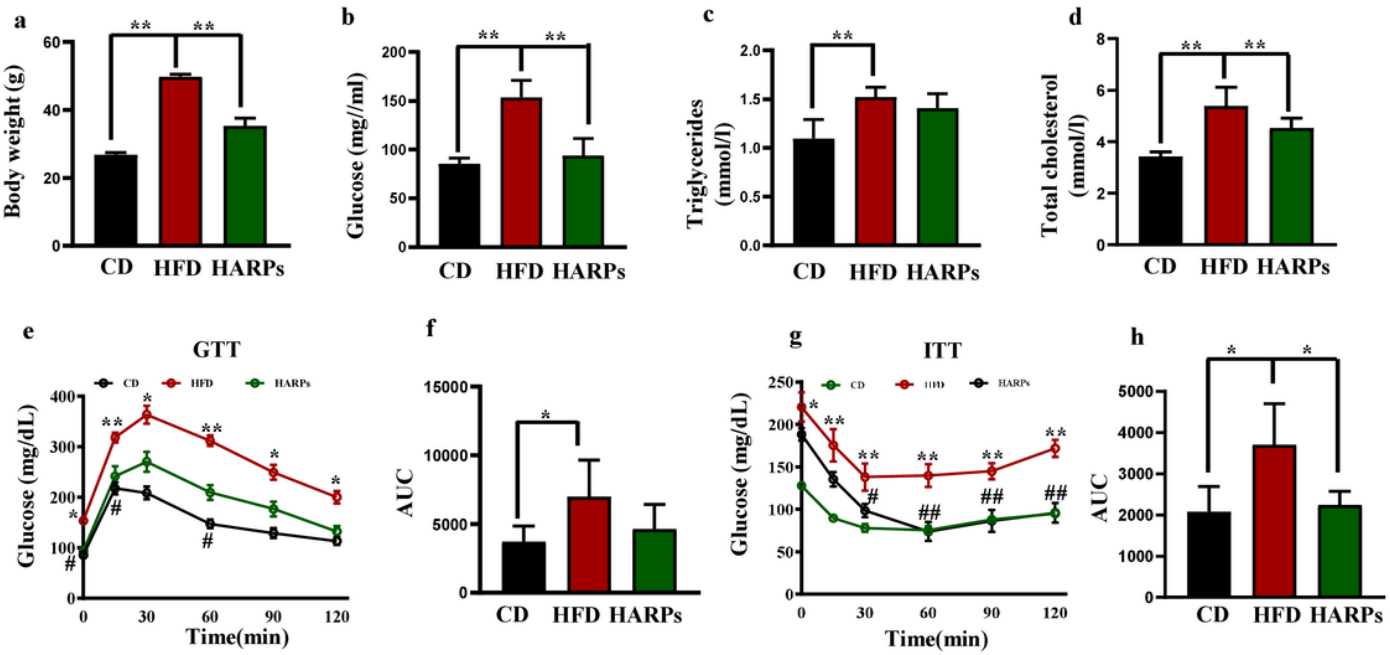

Figure 1

ARPs improved glucose and lipid metabolism disorders caused by HFD. (a) Body weight, (b) Glucose, (c) Triglyceride, (d) Total cholesterol, (e) GTT test 12-weeks after HFD induction, (f) The AUC of blood glucose in the GTT test, (g) ITT test 9-weeks after HFD induction, (h) The AUC of blood glucose in the ITT test. Data are presented as mean ± SEM, n=6. *p < 0.05, **p < 0.01, HARPs compared with HFD. ARPs,Anoectochilus Roxburghii Polysaccharides, GTT, intraperitoneal glucose tolerance test, HFD, high-fat diet, HARPs, HFD + ARPs, AUC, area under the curve.

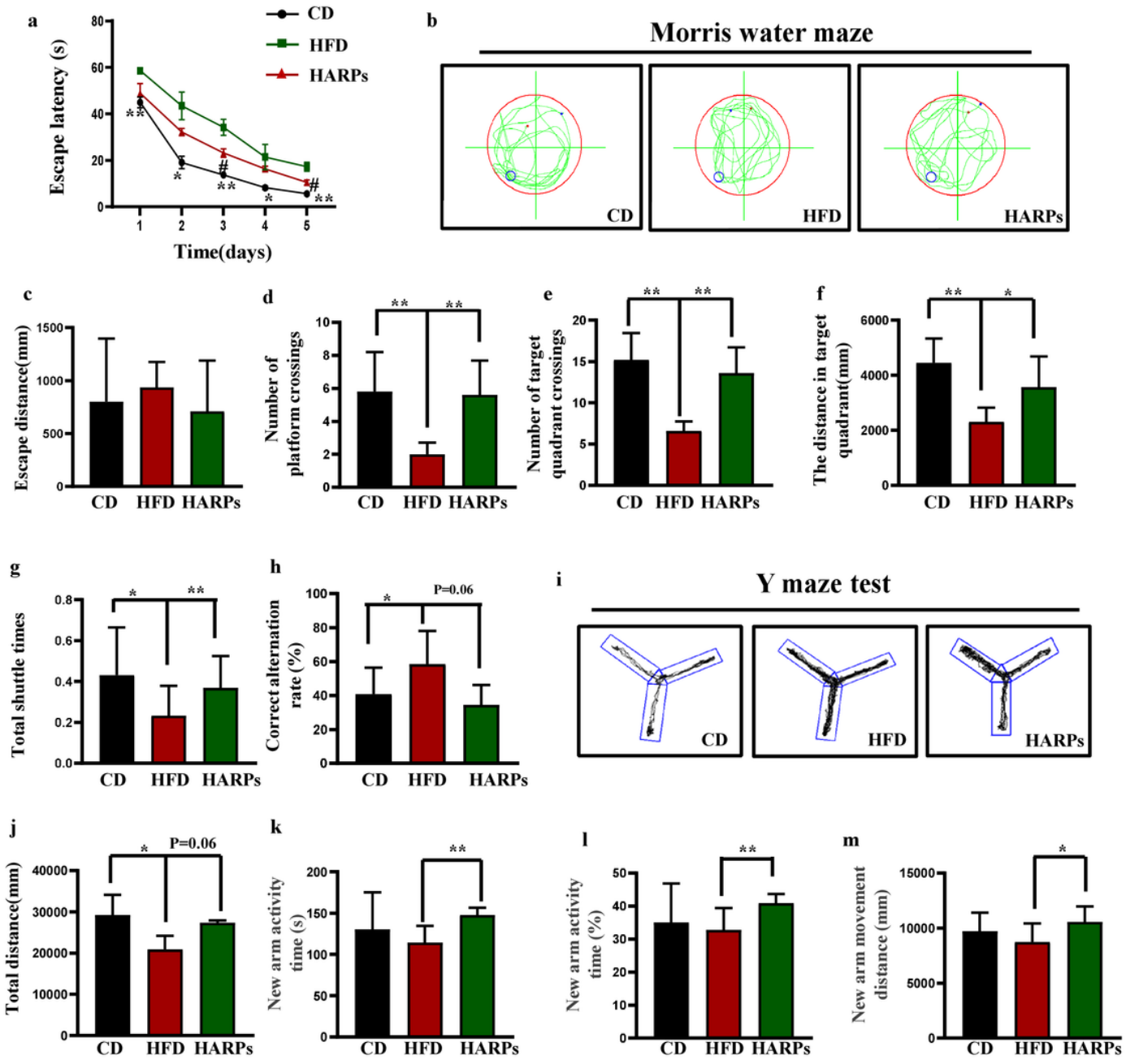

Figure 2

APPs ameliorated memory impairments triggered by HFD. (a) Escape latency time to reach the hidden platform were recorded during the 5 test days, (b) Morris water maze, (c) Escape distance, (d) Number of platform crossings, (e) Number of target quadrant crossings, (f) The distance in target quadrant of mice in the Morris water maze tests were recorded, (g) Total shuttle times, (h)Correct alternation rate, (i) Y-maze, (j) Total distance (mm), (k) New arm activity time (s), (l) New arm activity time (%), (m) New arm movement distance (mm). Data are presented as mean ± SEM, n=6 *p< 0.05, **p< 0.01, HARPs compared with HFD. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs

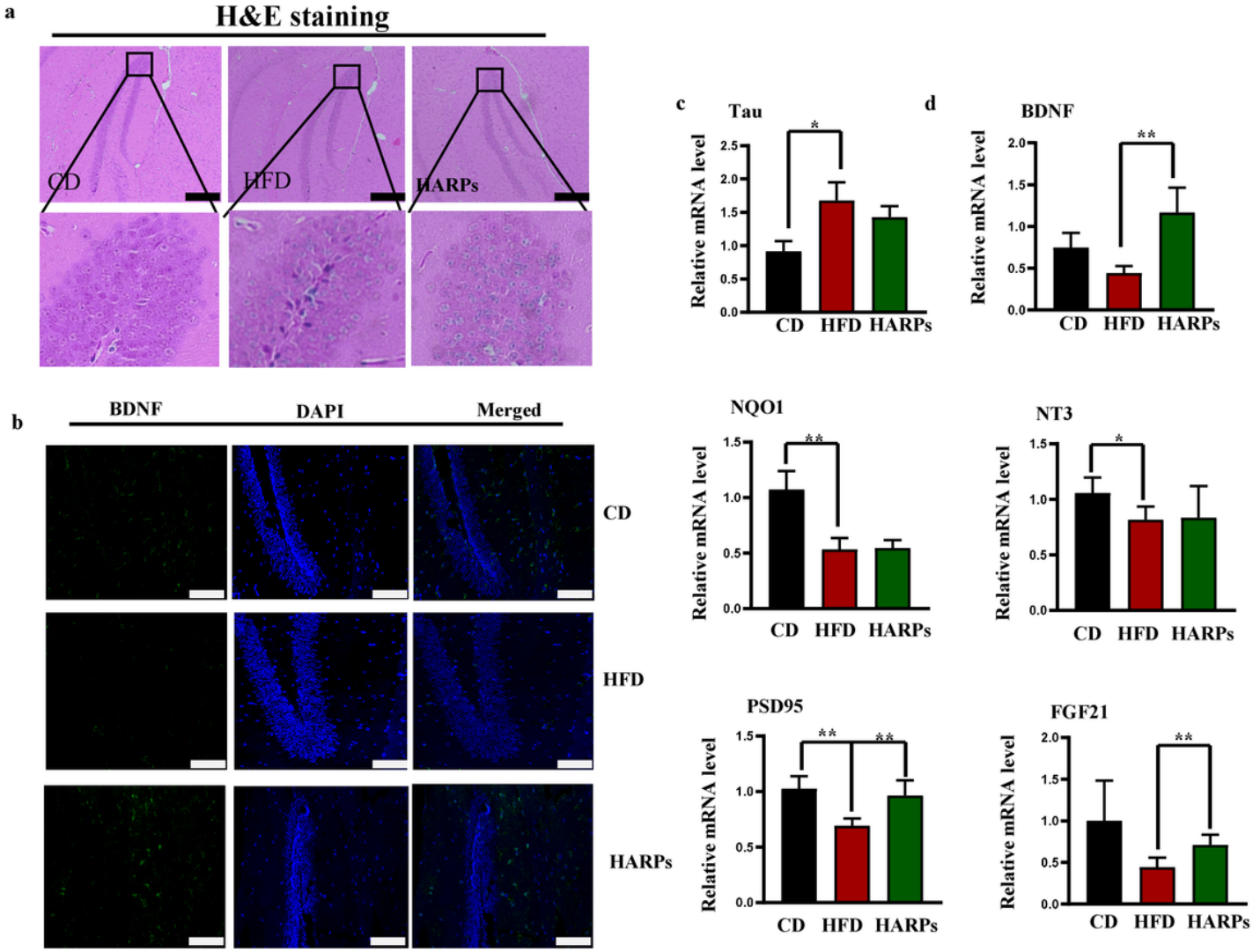

Figure 3

ARPs improved cognitive disorder induced by HFD. (a) H&E staining of hippocampus, (b) Immunofluorescence staining of BDNF in hippocampus, (c)The mRNA levels of BDNF and Tau in mice hippocampus, (d) the mRNA levels of NQO1, NT3, PSD95 and FGF21 in mice hippocampus. Data are presented as mean ± SEM, n=5 *p< 0.05, **p< 0.01. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs.

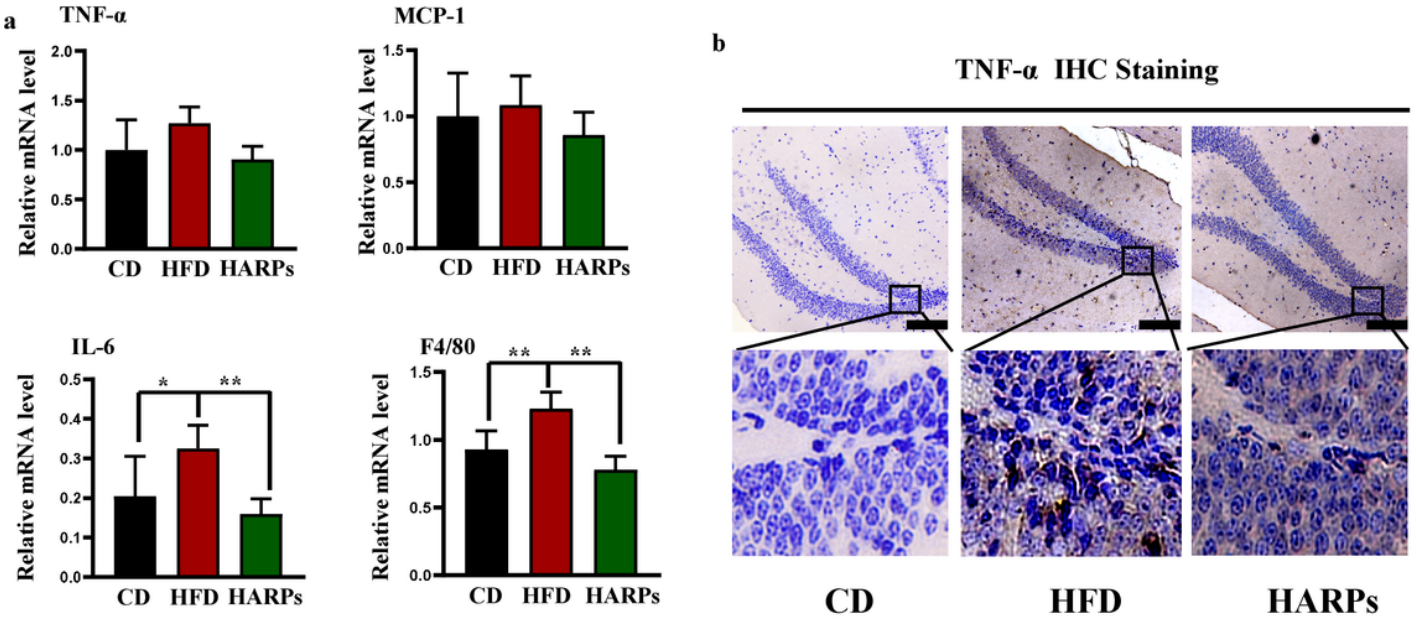

Figure 4

ARPs attenuated HFD-induced neuroinflammation in hippocampus. (a)the mRNA levels of TNF-α, MCP-1, IL-6 and F4/80 in mice hippocampus, (b) Immunohistochemical images of inflammatory activation marker TNF-α. Data are presented as mean ± SEM, n=5 *p< 0.05, **p< 0.01. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs.

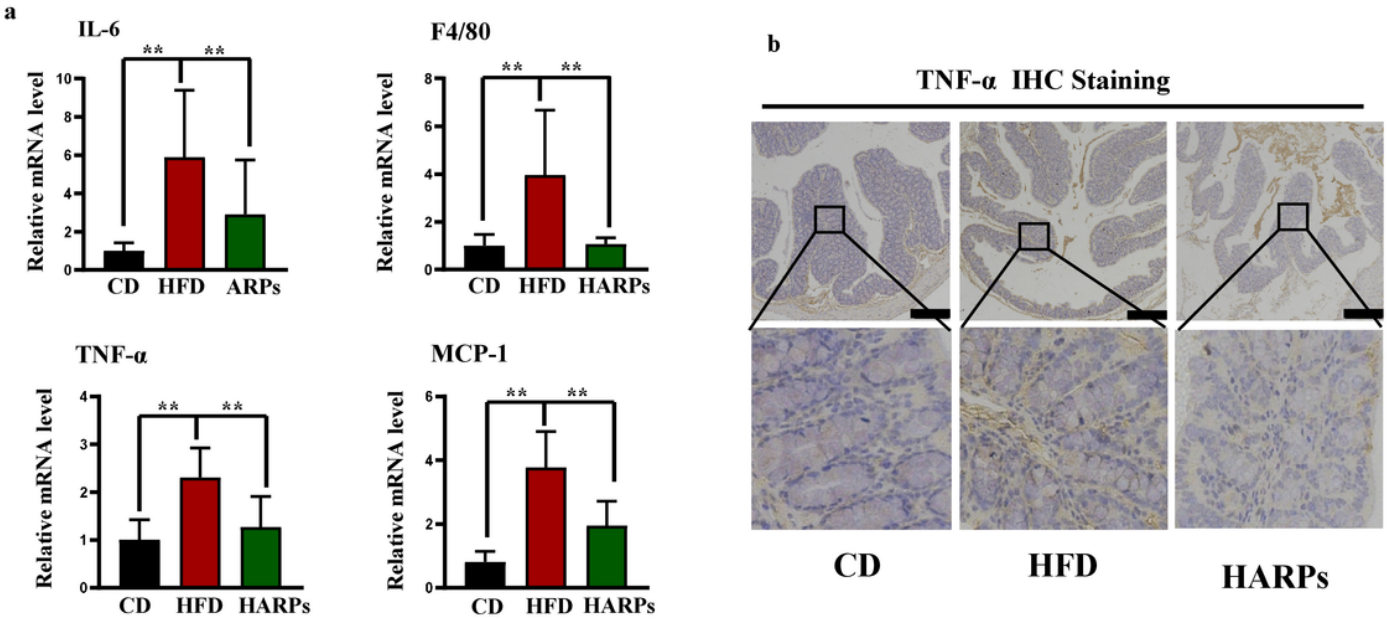

Figure 5

ARPs attenuated HFD-induced colon inflammation. (a) the mRNA levels of TNF-α, MCP-1, IL-6 and F4/80 in colon, (b) Immunohistochemical images of inflammatory activation marker TNF-α. Data are presented as mean ± SEM, n=5 *p< 0.05, **p< 0.01. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs.

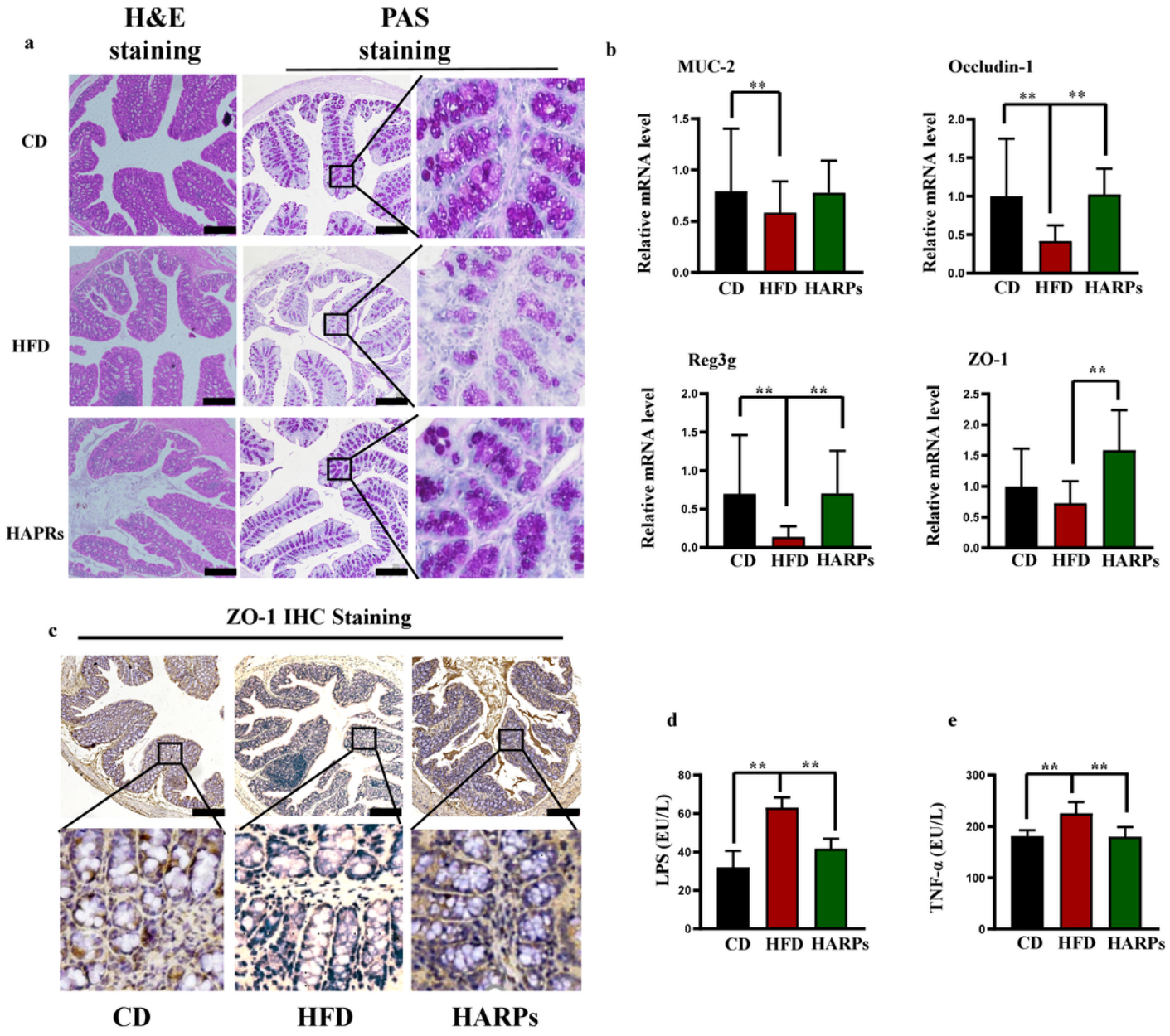

Figure 6

ARPs ameliorates intestinal barrier and microbial metabolite disorder induced by HFD. (a) H&E staining and PAS staining of colon, (b) the mRNA levels of MUC-2、Occludin-1、Reg3g and Zo-1 in colon, (c) Immunohistochemical images of ZO-1, (d) Serum LPS levels, (e) Serum TNF-α levels. Data are presented as mean ± SEM, n=5 *p< 0.05, **p< 0.01. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs, H&E, hematoxylin-eosin. PAS, Periodic Acid-Schiff.

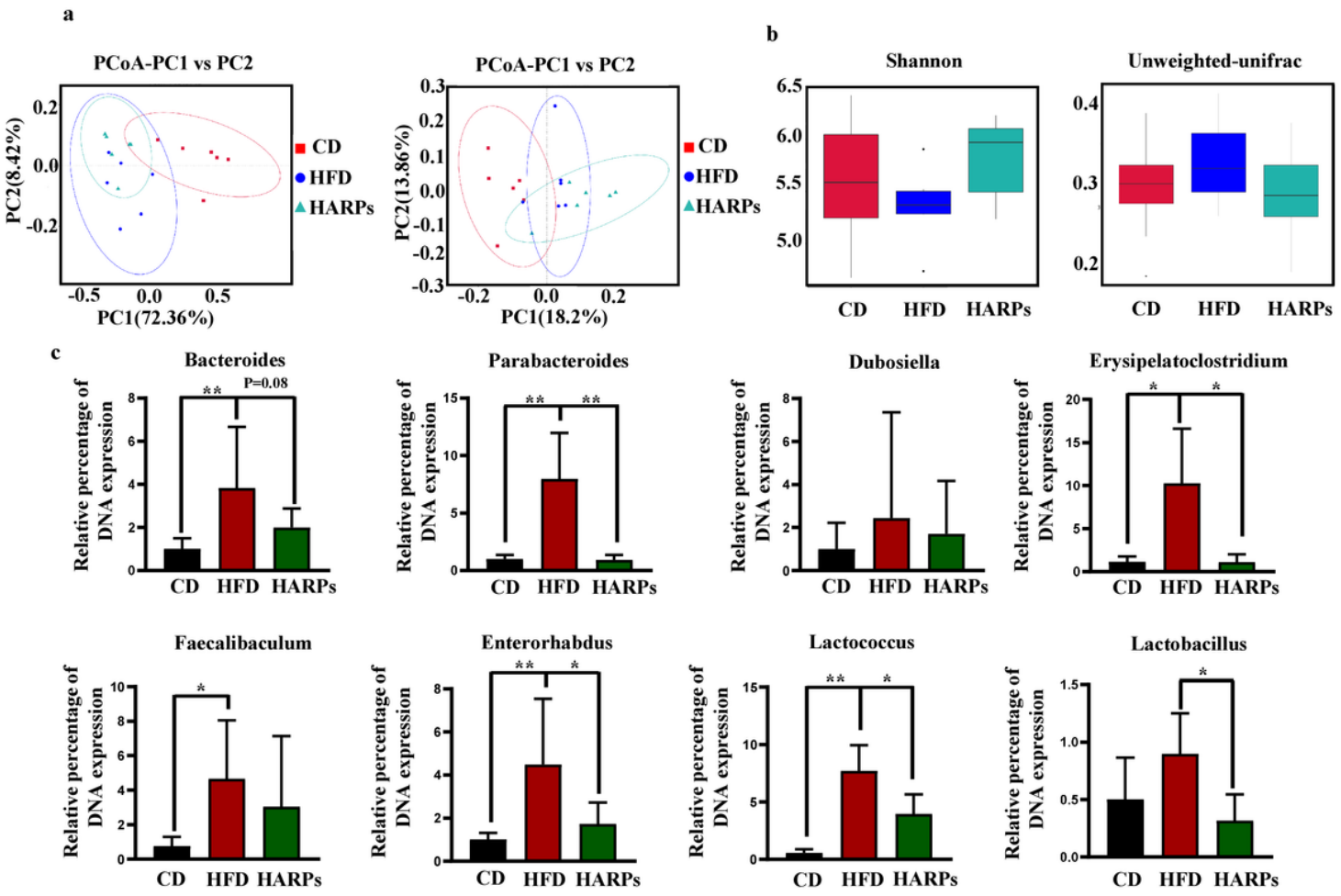

Figure 7

ARPs ameliorates HFD-induced intestinal microbial disturbance. (a) PCoA show the beta diversity of microbial communities, (b) the Unweighted-unifrac and Shannon analysis on OTUs (n=5), (c) Relative percentage of DNA expression of Parabacteroides, Enterorhabdus, Lactoccus, Bacteroides, Lactocbacllus, Erysipelatoclostridium, Dubosiella, Faecalibaculum. Data are presented as mean ± SEM, n=6 *p< 0.05, **p< 0.01. ARPs, Anoectochilus Roxburghii Polysaccharides. HFD, high-fat diet, HARPs, HFD + ARPs. PCoA, principal component analysis, operational taxonomic units (OTUs).

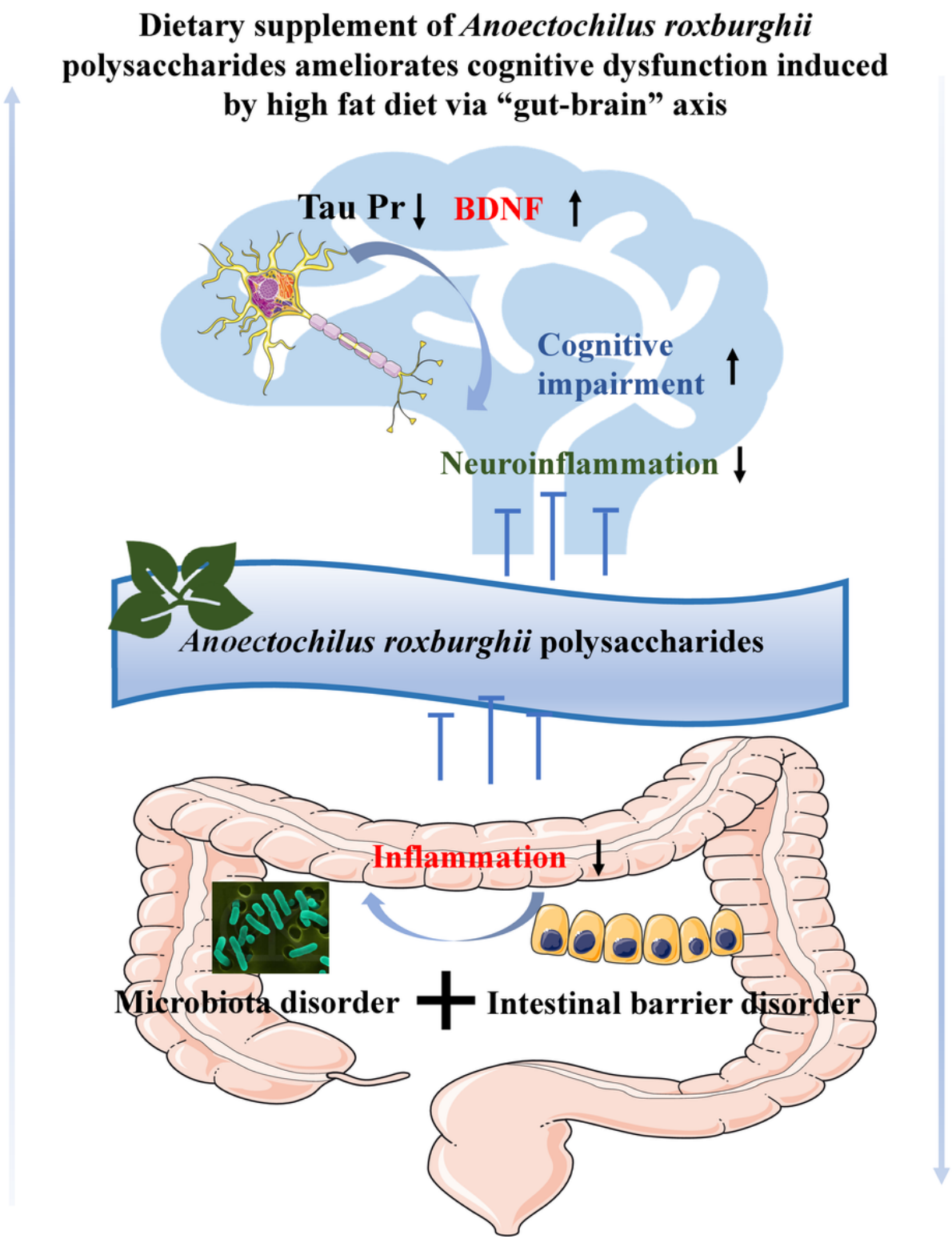

Figure 8

Graphic abstract ARPs increased the expression of BDNF and improved neuroinflammation, and ARPs supplementation restored intestinal barrier integrity and restored HFD-induced gut microbial dysbiosis.