Flavonoid-Amyloid Fibril Hybrid Hydrogels for Obesity Control via Construction of Gut Microbiota

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Experimental Section

Materials: Lysozyme derived from hen eggs (HEWL, L-6876) and Bis-Tris (14880) were purchased from Sigma-Aldrich Chemical Co., Ltd (St. Louis, MO, USA). Flavonoid samples with purities of greater than 98%, including EGCG, TF, TF-3, TF-3' and TF-33', were obtained from Chengdu Purify Co., Ltd. The commercially available kits used to determine the contents of TC and LDL-C in serum; the ELISA kits used to measure the levels of TNF-α, IL-6, and IL-1β in serum; the ELISA kits used to determine the levels of COMT in the liver; and the commercially available kits used to determine the hepatic levels of malondialdehyde (MDA), myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The hepatic level of NO was determined with commercially available kits obtained from Beyotime Biotechnology (Shanghai, China). The level of CRP in serum was measured using commercial ELISA kits obtained from Joyee Biotechnics Co., Ltd (Anhui, China). A MiniBEST Universal RNA Extraction Kit was purchased from TaKaRa Bio. Inc. (Beijing, China).

Formation of Lysozyme Amyloid Fibrils: First, the commercially obtained HEWL protein was dissolved at 10 wt% and dialysed as described before.[1] After lyophilization, the purified lysozyme monomers were dissolved at 2 wt% in pH 2 solution and were further thermally treated in a 90 °C oil bath for 8 hours with agitation, during which the amyloid fibrils were fabricated.

Interaction Between Flavonoids and Amyloid Fibrils: The affinity kinetics of the

flavonoids (EGCG, TF, TF-3, TF-3', TF-33') for the lysozyme amyloid fibrils were determined using a ForteBio Octet RED96 system (ForteBio). As described before, [2] the amyloid fibrils were biotinylated and were then purified. Then, the biotinylated amyloid fibrils were attached to the surface of streptavidin-coated sensors (SA, ForteBio). [2] SA sensors coated with amyloid fibrils were transferred to flavonoid solutions of different concentrations and were then transferred to the working solution to exclude non-specific binding. The concentrations of EGCG, TF-3, TF-33' and TF-3' were 5455, 2728, 1364, 681.9, 340.9, 170.5, or 85.2 nM, and the concentrations of TF were 10,900, 5455, 2728, 1364, 681.9, 340.9, or 170.5 nM. Data analysis and KD value calculation were performed as described in the previous study. [2]

Mixture of Flavonoids with Lysozyme Amyloid Fibrils: The flavonoid (EGCG, TF, TF-3, TF-3' or TF-33') solution at different contents from 0.05 to 0.5 wt% was prepared with dissolution in 10 mM Bis-Tris buffer (pH 6.8). The solubility of EGCG in the aqueous buffer was as high as 8.0 wt%. However, TF was quite difficult to dissolve and was excluded from the following study. The lysozyme amyloid fibril solution (2 wt%, pH 2) and the flavonoid (EGCG, TF, TF-3, TF-3' or TF-33') solution were mixed at equal volumes with magnetic agitation (30 s), resulting in a mixture with a final flavonoid concentration in the range of 0.025 to 0.25 wt%. In the case of EGCG, the flavonoid concentration was as high as 4.0 wt%. All samples had a pH value of 3.0. An equal volume of Milli-Q water (pH 2) was blended with Bis-Tris buffer as the blank working solution. Hydrogel formation was preliminarily assessed by the upturn test. The viscoelastic properties of the formed hydrogels containing the different types of

flavonoids at various concentrations were studied by rheological measurements. Observation of the samples under polarized light was performed to assess birefringence.

Rheological Measurements: As described in our previous study, [2] oscillatory rheological experiments were conducted with a Physica MCR302 shear rheometer (Anton Paar, Austria).

Cryo-TEM Experiments: After slight shaking of the flavonoid-amyloid fibril hydrogels to form a flowing viscous liquid, 2.5 μL sample was withdraw and deposited on a glow-discharged holey carbon grid (Quantifoil R1.2/1.3), and vitrification was performed with a Vitrobot Mark IV (Thermo Fisher Scientific). A Talos F200C equipped with a Ceta 4k × 4k camera was applied for the Cryo-TEM imaging in the Cryo-Electron Microscopy Center, Zhejiang University (Hangzhou, China). The camera was operated at an accelerating voltage of 200 kV at nominal magnifications of 73,000×, 92,000× and 120,000×.

Cryo-SEM Experiments: The microstructural morphology of the flavonoid-amyloid fibril hydrogels was visualized using a cryo-scanning electron microscope (SU8010, HITACHI, Japan) equipped with a Quorum PP3010T sample transfer channel. The hydrogel sample (~20 μL) was mounted on a copper holder and quickly frozen in liquid nitrogen slush (-210 °C). Then, the sample was etched at -140 °C and held at that temperature for 10 min of sublimation, returned to -175 °C consequent with coating of sputtered gold (10 mA, 90 s). SEM imaging was performed using an Everhart Thornley detector (ETD) and a solid-state detector (SSD).

Mouse Model of Obesity Induced by Feeding with A HFD, Animals and

Treatments: Mice were acclimatized for 7 days before further treatments. The room temperature was maintained at 21 to 25 °C, the humidity was controlled at 70%-75%, and the lighting was set on a 12-hour light/dark cycle. During this period, mice were provided access to sterile water and a standard laboratory chow diet ad libitum. Then, forty mice were grouped as follows (8 mice per group): NC, HFD, HFD + hydrogels (O-H), HFD + EGCG (O-E) and HFD + fibrils (O-F). Mice in the HFD group were fed a HFD every day for 8 weeks, inducing abnormal weight gain. In addition to daily feeding with a HFD, mice in the O-H, O-E and O-F groups were also treated with EGCG-amyloid fibril hydrogels, EGCG and amyloid fibrils, respectively, by oral gavage (200 μL/day) for 8 weeks. The flavonoid dosage was 150 mg/kg/d; correspondingly the flavonoid content in hydrogel or solution samples was 1.5 wt%. The dosage of lysozyme amyloid fibrils was 100 mg/kg/d. Mice in the NC group were fed normal standard chow. The body weight and food intake of the mice were recorded daily. Fresh faecal samples were collected from each mouse at weeks 0, 2, 4, 6, and 8. The collected faeces were stored at −80 °C after snap freezing in liquid nitrogen. The faeces collected in days before the sacrifice of mice were also used to determine the lipid content according to the protocol for "Lipid Extraction from Mouse Feces". [3]

Blood collection from anaesthetized mice was performed via cardiac puncture in prior to the experiment end, the plasma was kept for 4 hours at room temperature. After centrifugation of the plasma for 20 min, 3000 rpm at 4 °C, the supernatant (serum) was removed and stored at −80 °C before analysis. The caecal contents and liver and adipose tissue samples collected in the experiment were first frozen in liquid nitrogen and then

stored at -80 °C. The intestinal region from the caecum to the anus was removed for length measurement. After washing in saline, the distal colon was stored at -80 °C.

Measurement of Lipid Indexes and Pro-inflammatory Cytokines in Serum: The serum levels of TC and LDL-C and factors for pro-inflammatory, including CRP, IL-1β, IL-6, and TNF-α, were quantified with commercially available kits.

Histological Characterization. Haematoxylin and Eosin (H&E) Staining: Fresh liver and adipose tissues were fixed with 4% paraformaldehyde for more than 24 hours and were then embedded in paraffin and sliced into 4 μm full-thickness sections. Then, the sections were stained with haematoxylin and eosin (H&E) to characterize histological changes. And the visualization of tissue was performed using a Nikon ECLIPSE TI-SR fluorescence microscope (Tokyo, Japan). The steatosis score of liver was evaluated based on the H&E staining results according to the method and algorithm established in a previous study.[4] The quantities of large or medium-sized lipid droplets, not including foamy microvesicles were assessed as the steatosis score (S), from 0 to 3 (S0: <5%; S1: 5%-33%, mild; S2: 34%-66%, moderate; S3: >67%, marked).[4] *Oil red O staining:* Frozen liver sections (10-15μm thick) were stained with Oil Red O (C0158, Beyotime Biotechnology, China) for 20 min, washed in distilled water for 20 s and then counter-stained with haematoxylin (C0107, Beyotime Biotechnology, China) for 1min. Sections were examined under light microscopy.

Colon, Liver and Adipose Tissue RNA Extraction and qRT-PCR Analysis: Total RNA in colon (20 mg) or liver (20 mg) was extracted using the MiniBEST Universal RNA Extraction Kit (TaKaRa Bio. Inc.). Total RNA from epididymal adipose tissue

(100 mg) was extracted using RNAiso plus reagent (TaKaRa Bio. Inc.). The RNA samples were checked for the purity and concentration with a UV-vis spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA). For all samples, the ratio of the absorbance at 260 nm to the one at 280 nm was calculated, which were all in the acceptable range of 1.8 to 2.0. Then, 500 ng RNA was reversely transcribed to prepare the complementary DNA (cDNA) with PrimeScript RT Master Mix (TaKaRa Bio. Inc.). Real-time PCR was conducted with the utilization of SYBR Green Fast Mix (Tsingke Bio. Inc., Beijing, China) in a QuantStudio 6 Flex system (Thermo Fisher Scientific Inc., USA). The detailed procedure was described below: an initial denaturation step at 95 °C for 1 min following with 40 cycles of 95 °C for 10 s, 60 °C for 5 s and 72 °C for 30 s. Relative expression of gene was quantified by the $2^{-\Delta\Delta Ct}$ method with normalization to the expression of the housekeeping gene GAPDH. The specific primers applied are listed in Table 1.

Table 1. Primer sequences utilized in the RT-qPCR experiments.

Target gene	Primer	Sequence (5′-3′)
LXRα	Forward	TCAGAAGAACAGATCCGCTTG
	Reverse	CGCCTGTTACACTGTTGCT
FAS	Forward	GGCACCTATGGCGAGGACTT
	Reverse	GCCCTCCCGTACACTCACTC
PPARγ	Forward	GCTGAACGTGAAGCCCATCG
	Reverse	GGCGAACAGCTGAGAGGACT
Slc27a4	Forward	TCCTCCACTGTTGGACCTTC
	Reverse	CCTGGGAGGAAGCAGAAAGA
CD36	Forward	GCCAAGCTATTGCGACATGA
	Reverse	GGCATTGGCTGGAAGAACAA
LPL	Forward	CATCGAGAGGATCCGAGTGAA
	Reverse	TGCTGAGTCCTTTCCCTTCTG
Fiaf	Forward	CAATGCCAAATTGCTCCAATT
	Reverse	TGGCCGTGGGCTCAGT

PPARα	Forward	TCATCAAGAAGACCGAGTCC
	Reverse	CCTCTTCATCCCCAAGCGTA
UCP-1	Forward	AGCTTTGCCTCACTCAGGAT
	Reverse	AGAGGCAGGTGTTTCTCTCC
CPT1	Forward	CATCCACGCCATACTGCT
	Reverse	GACCTTGAAGTAACGGCCTC
IL-1β	Forward	AGCTTCAAATCTCGCAGCAG
	Reverse	TCTCCACAGCCACAATGAGT
IL-6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
TNF-α	Forward	CTCATGCACCACCATCAAGG
	Reverse	ACCTGACCACTCTCCCTTTG
TLR4	Forward	GCTCTCAGCCATCCACAAAG
	Reverse	GAGTCGGGAAGAGGAAGAGG
MCP-1	Forward	AACTGCATCTGCCCTAAGGT
	Reverse	CTGTCACACTGGTCACTCCT
ZO-1	Forward	CAGAGGTGAAGGACCACCAT
	Reverse	GGCTCCTTCCTGTACACCTT
Occludin-1	Forward	TCGCCATATTTGCCTGTGTG
	Reverse	CCAAAGAGCCCTGTCCCATA
NFIL3	Forward	GACAGCGAGTTTGAAGGCAT
	Reverse	CGTCACCTGCACTGAGAAAG
SCD-1	Forward	GGCTTCCAGATCCTCCCTAC
	Reverse	ACCCTCGCATTCAGTGGTTA
Claudin-1	Forward	AGCTGCCTGTTCCATGTACT
	Reverse	CTCCCATTTGTCTGCTGCTC
Claudin-5	Forward	TTCTTCTATGCGCAGTTGGC
	Reverse	TTGGTGCCTACTTCACCGAT
MUC-2	Forward	GCTCTCAAATGGTGCAGAGG
	Reverse	AGTGGGAAGGGATGAATGGG
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	TGTAGACCATGTAGTTGAGGTCA

Study on Gut Microbiota, Faecal DNA Extraction and the V3-V4 Region Sequencing:

Amplification of the 16S rRNA V3-V4 Gene Region: Microbial DNA was extracted from all faecal matter samples, which were stored at -20 °C and delivered on dry ice. DNA extraction was conducted using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) with the mechanical lysis of bacterial cells disrupted by bead beating. The final DNA samples were measured for concentration and purity with a NanoDrop 2000

UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and the DNA integrity was checked by electrophoresis on an agarose (1%) gel. The V3-V4 hypervariable regions of the bacterial 16S rRNA gene were amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by PCR in a thermocycler (GeneAmp 9700, ABI, USA). PCR was performed according to following program: an initial denaturation at 95 °C for 3 min, followed by 27 cycles of 95 °C for 30 s, annealing at 55 °C for 30 s, consequent elongation at 72 °C for 45 s, and a final extension step at 72 °C for 10 min. PCRs were conducted for three times in a 20 μL mixture containing FastPfu Buffer (5 ×, 4 μL), dNTPs (2.5 mM, 2 μL), each primer (5 μM, 0.8 μL), FastPfu Polymerase (0.4 μL) and template DNA (10 ng). The resulted products from PCR were extracted from an agarose gel (2%) and further purified by an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluorTM-ST (Promega, USA) in line with the manufacturer's protocol. Purified amplicons were pooled in equimolar amounts and subjected to paired-end sequencing (2 × 300) by Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China) on an Illumina MiSeq platform (Illumina, San Diego, USA) in line with the standard protocols.

Analysis of 16S rRNA V3-V4 Sequencing Data: Demultiplex and quality filter with Trimmomatic, as well as merging with FLASH were applied to process raw fastq files according to the criteria below: First, reads were truncated across any site where an average quality score of less than 20 over a 50 bp sliding window. Second, primers

were exactly matched with an allowance of two nucleotide mismatches, the reads having ambiguous bases were deleted. Third, merging was performed on the sequences with an overlap of more than 10 bp, according to the overlap sequence. A similarity of 97% was set as the cutoff for OTUs cluster using UPARSE (version 7.1 http://drive5.com/uparse/), and UCHIME was used to identify and remove chimeric sequences. The taxonomy of each 16S rRNA gene sequence was analysed by the RDP Classifier algorithm against the Silva (SSU123) 16S rRNA database using a confidence threshold of 70%.

The sequencing depth was normalized by reducing the number of sequences of all samples to 34,336. Mothur 1.30.2 was utilized for analysis of α diversity, including the Shannon index and Simpson index. Generation of abundance tables at each taxonomic level and calculation of the β diversity distance metric were conducted on the QIIME (1.9.1) platform. The significance of alterations in the gut microbiota was statistically analysed by PerMANOVA (999 permutations, $p < 0.001$) in R version 3.3.1. The fundamental input for PCoA and PerMANOVA was the Bray-Curtis distance data matrix. To identify OTUs with differences between groups, linear discriminant analysis (LDA) effect size (LEfSe) was carried out on OTUs with relative abundances of greater than 0.1%. The logarithmic LDA score for discriminative features was set at the threshold of 3.0, and 114 OTUs with differences between groups were obtained. Then, the corresponding heatmap was generated according to these 114 OTUs. The significance of differences between the HFD and NC, HFD and O-H, HFD and O-E, and HFD and O-F groups was analysed by Tukey's test.

Faecal suspension inoculum preparation and faecal microbiota transplantation: All the mice in each group of NC, HFD, O-H, O-E, and O-F in the "Mouse Model of Obesity Induced by Feeding with A HFD" were utilized as the donors. The faecal samples were collected from the donors on every morning for three consecutive days before the sacrifice of the donor mice. The faecal collected form individual mice in each group were mixed together, to avoid the different response to HFD caused by the differences in the microbiota composition of any individual mouse. [5, 6] Each fecal sample (0.5 g) was diluted in 5 mL of a 0.9% (w/v) sterile normal saline in an anaerobic chamber (80% N_2:10% CO_2:10% H_2). The fecal material was suspended by thorough vortexing (5 min) and centrifuged at 4 °C 300 rpm/min for 5 min. The clarified supernatant was transferred to a clean tube and used immediately for gut microbiota transplantation. Surveillance for bacterial contamination was performed by periodic bacteriological examinations of feces, food and padding. Normal saline was added into the samples with sufficient mixing. The mixtures were then cultured using the spread plate method on: 1) LB agar, Brain Heart Infusion agar and Thioglycolate agar under aerobic condition at 37°C for aerobic bacteria; 2) on Gifu anaerobic medium (GAM) agar under anaerobic condition at 37°C for anaerobic bacteria; and 3) on Modified Martin Agar and Tryptone Soya agar under aerobic condition at 25-28°C for fungi. All cultures were examined under optical microscope after 1, 2, 4, 7 and 14 days.

Forty germ-free male C57BL/6J mice were kept in plastic isolators with flexible-film and a regular 12-h light cycle was used. Lights were on at 06:00. A sterilized normal chow diet containing 10% energy from fat (3.25 kcal/g, SLAC) was applied to

feed the mice. The germ-free mice were housed in individual cages at age of 4 weeks, which were randomly divided into five groups (each group was maintained in an individual isolator). After acclimatization for one week, the five groups of mice (n = 8, per group) were orally gavaged with the faecal suspension inoculum (100 μL) taken from each of the donor group described above, which were named NC-FMT, HFD-FMT, O-H-FMT, O-E-FMT and O-F-FMT groups, respectively. The germ-free mice transplanted with gut microbiota in each group were fed a HFD every day for 4 weeks. The body weight and food intake of the mice were recorded daily. Fresh faecal samples were collected from each mouse at week 4 before the end of the experiment. The collected faeces were stored at −80 °C after snap freezing in liquid nitrogen. And the following methods for serum preparation, organ tissues collection and storage, measurement of lipid indexes and pro-inflammatory cytokines in serum, colon tissue RNA extraction and qRT-PCR analysis, 16S rRNA V3-V4 Sequencing and data analysis were conducted as described above in the sections of SPF mice experiment.

Long-term Toxicity Study: The experiment was performed according to the National Laboratory Animal Welfare Guidelines and Animal Experiment Ethics. Six-week-old male ICR mice weighing approximately 32 g were obtained from SLAC Laboratory Animal Co., Ltd (Shanghai, China). In prior to the experiment, all mice were cared for and fed as described in the section of "Mouse Model of Obesity Induced by Feeding with A HFD". Forty-eight mice were grouped (8 mice per group) by treatment as follows: NC; EGCG-amyloid fibril hydrogels with EGCG concentrations of 1 wt% (H-L), 2 wt% (H-M) and 4 wt% (H-H); and amyloid fibril solution with fibril

concentrations of 1 wt% (F-L) and 2 wt% (F-H). Mice in the H-L, H-M and H-H groups were administered EGCG-amyloid fibril hydrogels containing EGCG concentrations of 1 wt%, 2 wt% and 4 wt%, respectively, by oral gavage (200 μL/day) for 9 weeks. The corresponding dosages of the flavonoid in these samples used for the animal experiments were 100, 200 and 400 mg/kg/day. The dosage of the amyloid fibrils was 100 mg/kg/d. Mice in the F-L and F-H groups were treated with amyloid fibril solutions with fibril concentrations of 1 wt% and 2 wt%, respectively. The dosages of pure amyloid fibrils were 100 and 200 mg/kg/day, respectively. Mice in the NC group were fed purified water. The data recorded during the feeding of mice, sacrifice, blood collection and sample processing were treated as described in the section of "Mouse Model of Obesity Induced by Feeding with A HFD". The heart, liver, spleen, kidney, lung, thymus, pancreas, brain and testis were collected from mice. These organs and tissues were first weighed and then put into centrifuge tubes containing fixation solution.

Organ Coefficient: The ratio of the organ weight (liver, lung, kidney, brain, spleen, heart, thymus, testis, or pancreas) to the corresponding mouse body weight was calculated and recorded as the organ coefficient.

Indicators of Hepatic Toxicity: Homogenization of weighed liver samples mixed with physiological saline was performed in an ice-water bath. The MDA, nitric oxide (NO) and GSH contents as well as the CAT, MPO and SOD levels were measured by MDA, NO, GSH, CAT, MPO and SOD kits.

Routine Blood Parameters: Routine blood parameters, red blood cell count (RBC),

red blood cell distribution width coefficient (RDWC), red blood cell distribution width standard deviation (RDWS), haematocrit (HCT%), haemoglobin (HGB), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell count (WBC), mean platelet volume (MPV), platelet distribution width (PDWS), monocyte% (MON%), monocyte count (MON), neutrophil percentage (NEU%), neutrophil count (NEU), and lymphocyte percentage (LYM%) were measured using a Mindray instrument (BC-5000 Vet).

Serum Biochemical Indexes: Serum biochemical indexes of mice—alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total protein (TP), alkaline phosphatase (AKP), albumin (ALB), cholesterol (TC), total triglycerides (TG), glucose (GLU), IL-6, MCP-1, and creatinine (CRE) levels were quantified with the corresponding commercial kits for ALT, AST, BUN, TP, AKP, ALB, TC, TG, GLU, IL-6, MCP-1 and CRE.

Statistical Analysis: All data are shown as the mean ± standard deviation (SD) values. Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's test with SPSS software (IBM, U.S.A.). The level of statistical significance was set at $p < 0.001$, $p < 0.01$, $p < 0.05$. In the gut microbiota analysis, differences in the relative abundances of OTUs were calculated using Tukey's honestly significant difference (HSD) test. The level of statistical significance was set at $p < 0.05$.

Availability of Raw Data: The raw reads of 16S rRNA gene illumine sequence

were deposited into the NCBI Sequence Read Archive (SRA) database (accession number: SRP279880 for the SPF mice; accession number: SRP331994 for the germ-free mice with faecal microbiota transplantation).

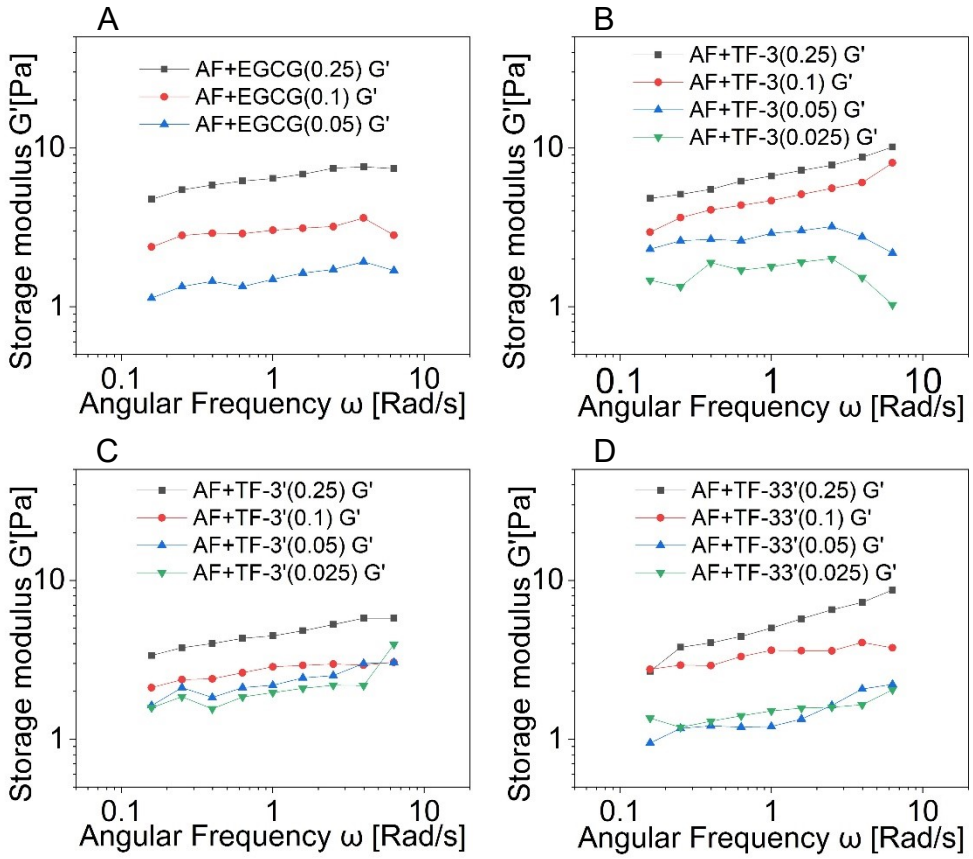

Fig. S1. Frequency dependence of the storage modulus (G') for the flavonoid-amyloid fibril hydrogels, which were performed at a fixed strain amplitude of $\gamma = 1\%$.

Fig. S2. The characterization of the EGCG-amyloid fibril hydrogels. (A) The photography of lysozyme amyloid fibrils mixed with different contents of EGCG：0, 0.1, 0.25, 0.5, 1, 2, 4 wt%. The mixture of the amyloid fibrils and EGCG with content ranging from 0.1 to 4 wt% formed the hydrogels which could hold themselves as the bottoms were placed upside down. Cryo-TEM images of the amyloid fibrils (1.0 wt%) mixed 2 wt% (B) and 4 wt% (C) EGCG.

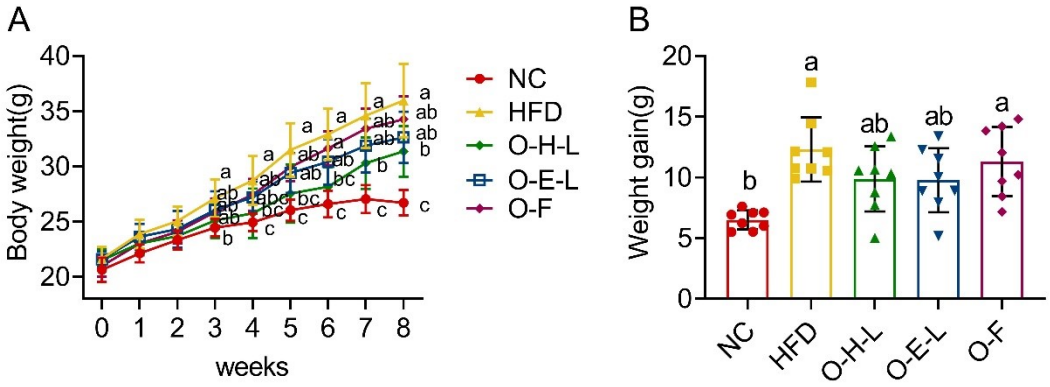

Fig. S3. Effect of oral administration of the EGCG-amyloid fibril hydrogels (O-H-L group), EGCG (O-E-L group) and amyloid fibrils (O-F group) on the body weight (A), weight gain (B) of the mice fed with high-fat diet (HFD group). The Mice fed with normal chow was used as the control (NC group). The EGCG content in the samples was 1.0 wt%.

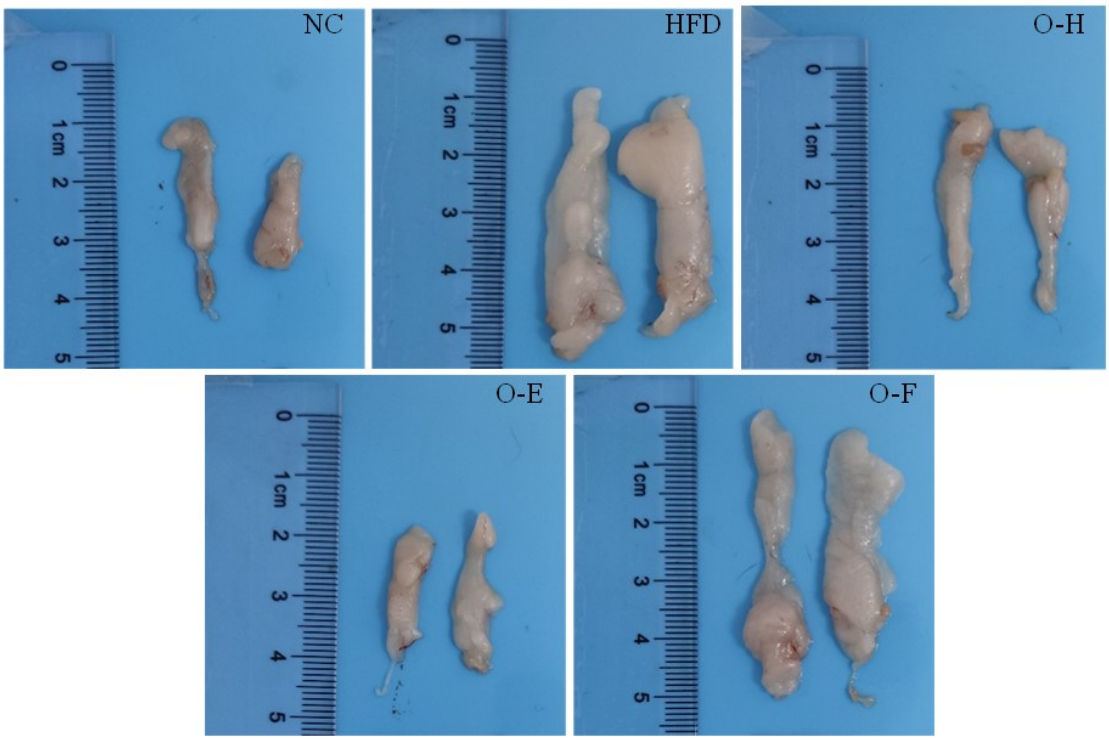

Fig. S4. The representation pictures of the epididymal adipose tissue in NC, HFD, O-H, O-E and O-F groups.

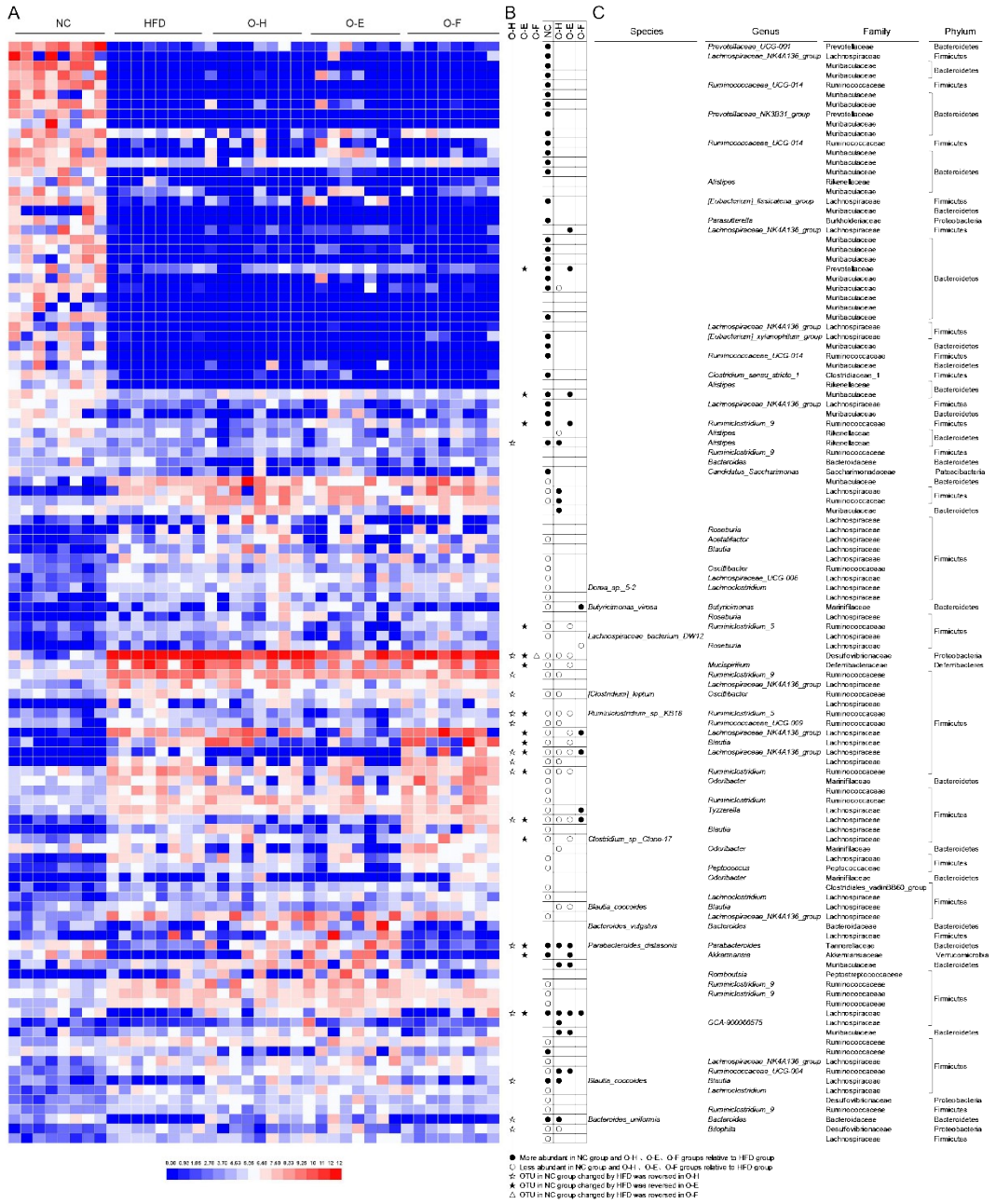

Fig. S5. Regulation of the gut microbial composition. (A) The relative abundances of 114 OTUs in different groups based on LDA shown as heatmap. The gut microbiota composition (NC) was altered by HFD feeding. Orally treating with EGCG-amyloid fibril hydrogels (O-H), EGCG (O-E) and amyloid fibrils (O-F) modulated the gut microbiota composition in mice fed a HFD. (B) The change direction of OTUs induced by HFD feeding and intervention with hydrogels (O-H), EGCG (O-E) or amyloid fibrils (O-F). The dots (●) and circles (○) represent the upregulation and the downregulation of the relative abundances of OTUs in NC, O-H, O-E or O-F groups in comparison to the HFD group. The hollow stars (☆), solid stars (★) and hollow triangles (△) indicate that the alterations in the OTUs in the NC group induced by HFD feeding were reversed by treatment with the hydrogels (O-H), EGCG (O-E) and amyloid fibrils (O-F),

respectively. (C) The OTUs represent bacterial taxonomic information: phylum, family, genus, and species. Differences in the relative abundances of OTUs were calculated using Tukey's HSD test. A value of $p < 0.05$ was considered significant.

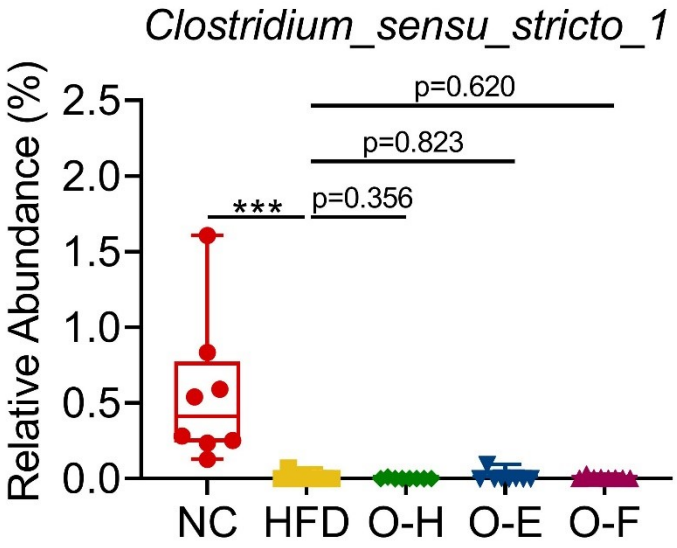

Fig. S6. Relative abundances of *Clostridium_sensu_stricto_1*. Data were shown in the form of mean ± standard deviation (SD). One-way ANOVA followed by Tukey post hoc test.

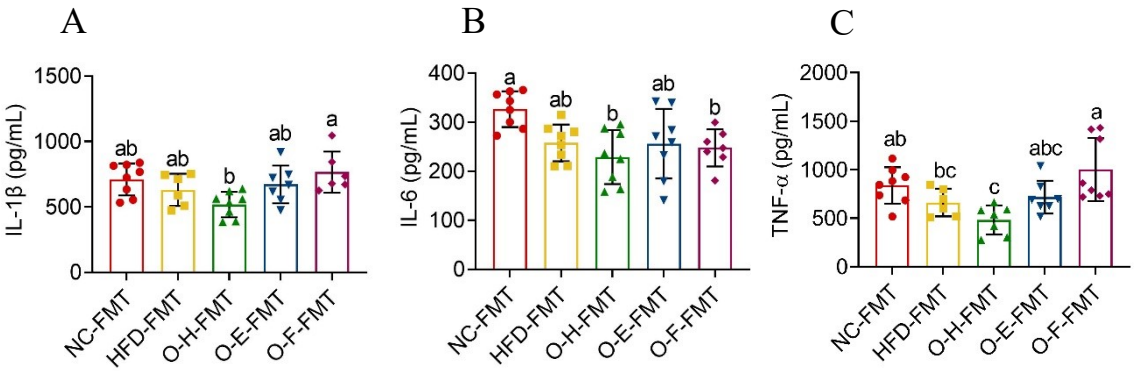

Fig. S7. Serum level of IL-1β (A), IL-6 (B) and TNF-α (C) in the germ-free mice transplanted with the gut microbiota from NC (NC-FMT), HFD (HFD-FMT), O-H (O-H-FMT), O-E (O-E-FMT) and O-F (O-F-FMT) groups.

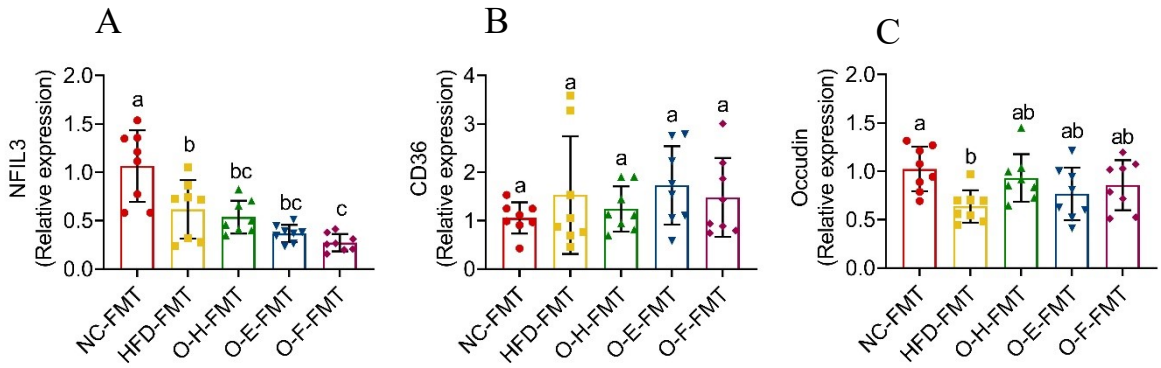

Fig. S8. Inhibition of lipid absorption genes in the gut of germ-free mice with facal microbiota transplantation (FMT). Relative expression levels of the genes controlling lipid absorption in the gut of the germ-free mice with FMT, *NFIL3* (A) and *CD36* (B); Relative expression level of the genes encoding tight junction proteins in the gut of the germ-free mice with FMT such as *Occudin* (C). The data are shown as the mean ± SD values; n=8. Significant differences are denoted by the different letters, $p<0.05$. One-way ANOVA followed by Tukey's test.

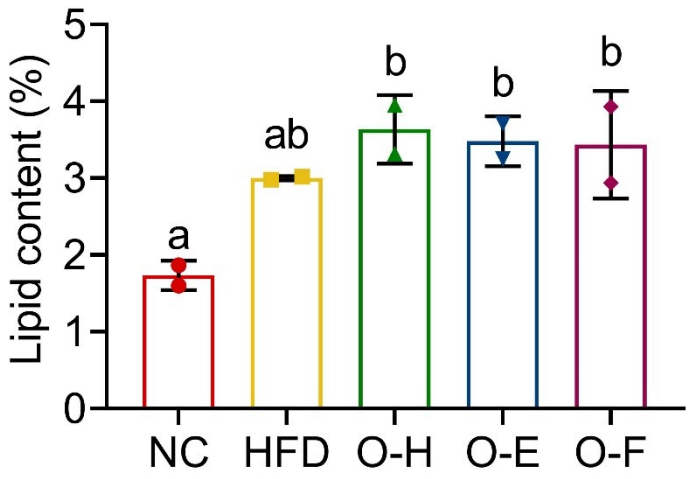

Fig. S9. The lipid composition of the faecal material in different groups of NC, HFD, O-H, O-E and O-F.

Toxicity Evaluations of Oral Administration of the Hydrogels

After oral treatment of the ICR mice for 9 weeks with the flavonoid-amyloid fibril hydrogels with EGCG contents of 1.0, 2.0 and 4.0 wt%, as well as the pure amyloid fibril solution with protein contents of 1.0 and 2.0 wt%, no obvious change could be observed in the body weight, food and water intake (Figure S7); the coefficient of different organs (Figure S8); the biochemical indexes in liver (Figure S9), the blood routing examination indexes (Figure S10), as well as serum biochemical indexes (Figure S11). These results indicated that no long-term toxicity could be observed in oral treatment with the flavonoid-amyloid fibril hydrogels with different EGCG contents and the amyloid fibril, in agreement with our earlier findings.

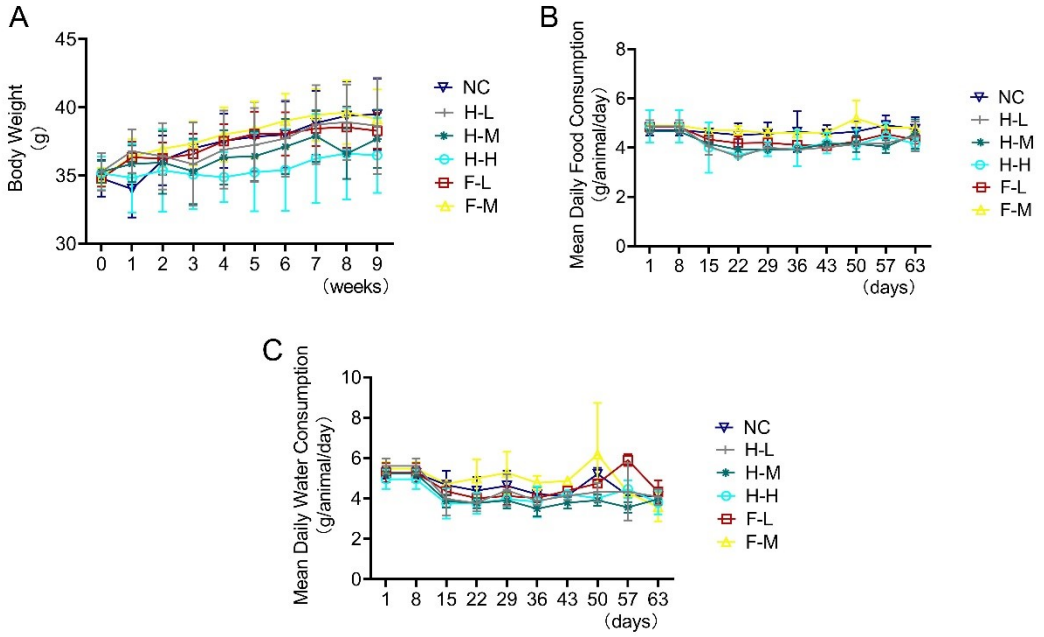

Fig. S10. The influence of oral administration of the EGCG-amyloid fibril hydrogels and the amyloid fibril solution on the body weight (A), daily food consumption (B) and daily water consumption (C) of normal mice for 9 weeks. The amount of the hydrogel or amyloid fibril sample fed to mice each time was 200 μL. The hybrid hydrogels had EGCG dosage of 100 mg/kg/day (H-L), 200 mg/kg/day (H-M) and 400 mg/kg/day (H-H). The amyloid fibril solution had the fibril does of 100 mg/kg/day (F-L) and 200 mg/kg/day (F-H).

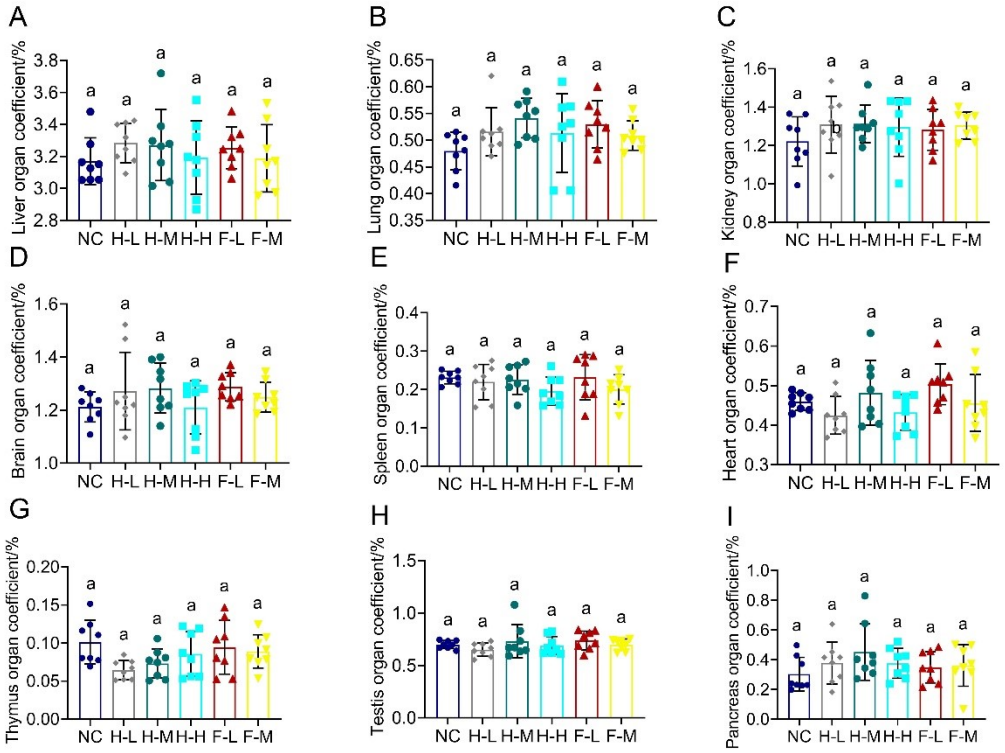

Fig. S11. The impact of oral administration of the EGCG-amyloid fibril hydrogels and the amyloid fibril solution on the organ coefficient of Liver (A), Lung (B), Kidney (C), Brian (D), Spleen (E), Heart (F), Thymus (G), Testis (H) and Pancreas (I) in mice after the 9 week experiment. The amount of the hybrid hydrogels and the amyloid fibril sample fed to mice was 200 μL each time. The hybrid hydrogels had the EGCG dosage of 100 mg/kg/day (H-L), 200 mg/kg/day (H-M) and 400 mg/kg/day (H-H). The amyloid fibril solution sample had the fibril content of 100 mg/kg/day (F-L) and 200 mg/kg/day (F-M).

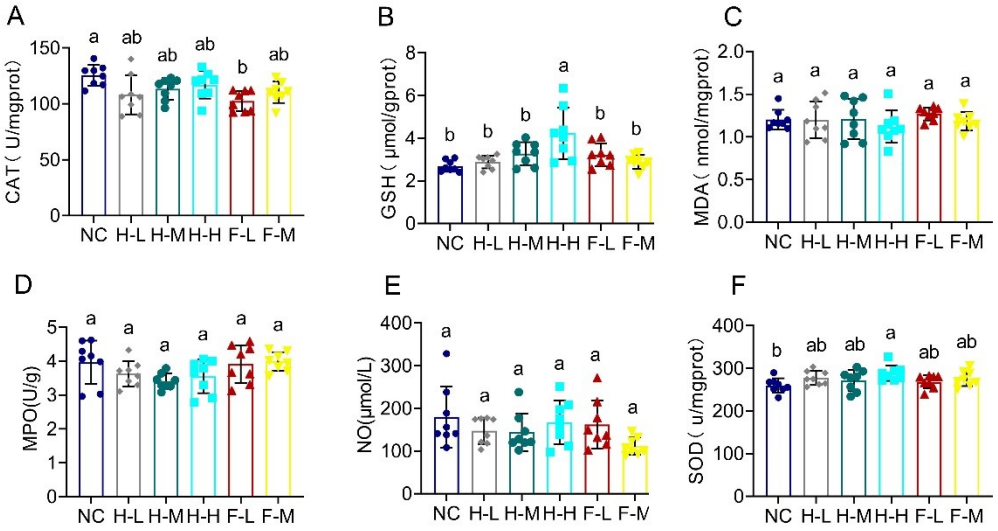

Fig. S12. The impact of oral administration of the EGCG-amyloid fibril hydrogels and the amyloid fibril solution on catalase (CAT, A), glutathione (GSH, B), malondialdehyde (MDA, C), Myeloperoxidase (MPO, D), nitric oxide (NO, E) and superoxide dismutase (SOD, F) in the liver of mice after the 9 week experiment. The amount of the hybrid hydrogels and the amyloid fibril sample fed to mice was 20 μL each time. The hybrid hydrogels had the EGCG dosage of 100 mg/kg/day (H-L), 200 mg/kg/day (H-M) and 400 mg/kg/day (H-H). The amyloid fibril solution sample had the fibril content of 100 mg/kg/day (F-L) and 200 mg/kg/day (F-M).

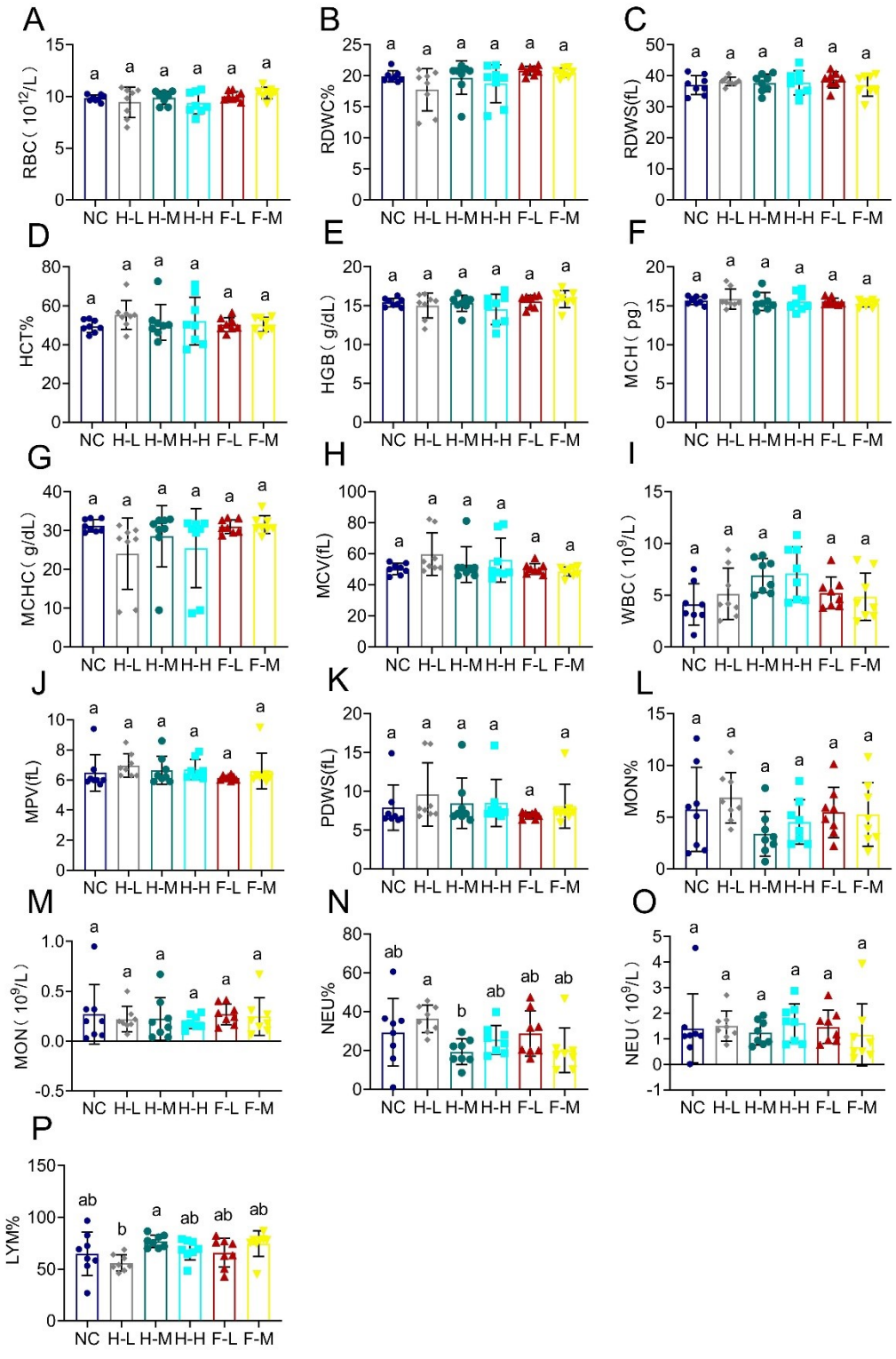

Fig. S13. The results of blood routine examination for the mice orally administrated with the EGCG-amyloid fibril hydrogels and the amyloid fibril solution samples: red blood cells (RBC, A), red blood cell distribution width coefficient (RDWC, B), red blood cell distribution width standard deviation (RDWS, C), hematocrit (HCT%, D), hemoglobin (HGB, E), mean corpuscular hemoglobin (MCH, F), mean corpuscular

hemoglobin concentration (MCHC, G), mean corpuscular volume (MCV, H), white blood cells (WBC, I), mean platelet volume (MPV, J), platelet distribution width (PDWS, K), monocyte% (MON%, L), monocyte (MON, M), neutrophils% (NEU%, N), neutrophils (NEU, O), lymphocyte% (LYM%, P). The amount of the hybrid hydrogels and the amyloid fibril sample fed to mice was 200 μL each time. The hybrid hydrogels had the EGCG dosage of 100 mg/kg/day (H-L), 200 mg/kg/day (H-M) and 400 mg/kg/day (H-H). The amyloid fibril solution sample had the fibril content of 100 mg/kg/day (F-L) and 200 mg/kg/day (F-M).

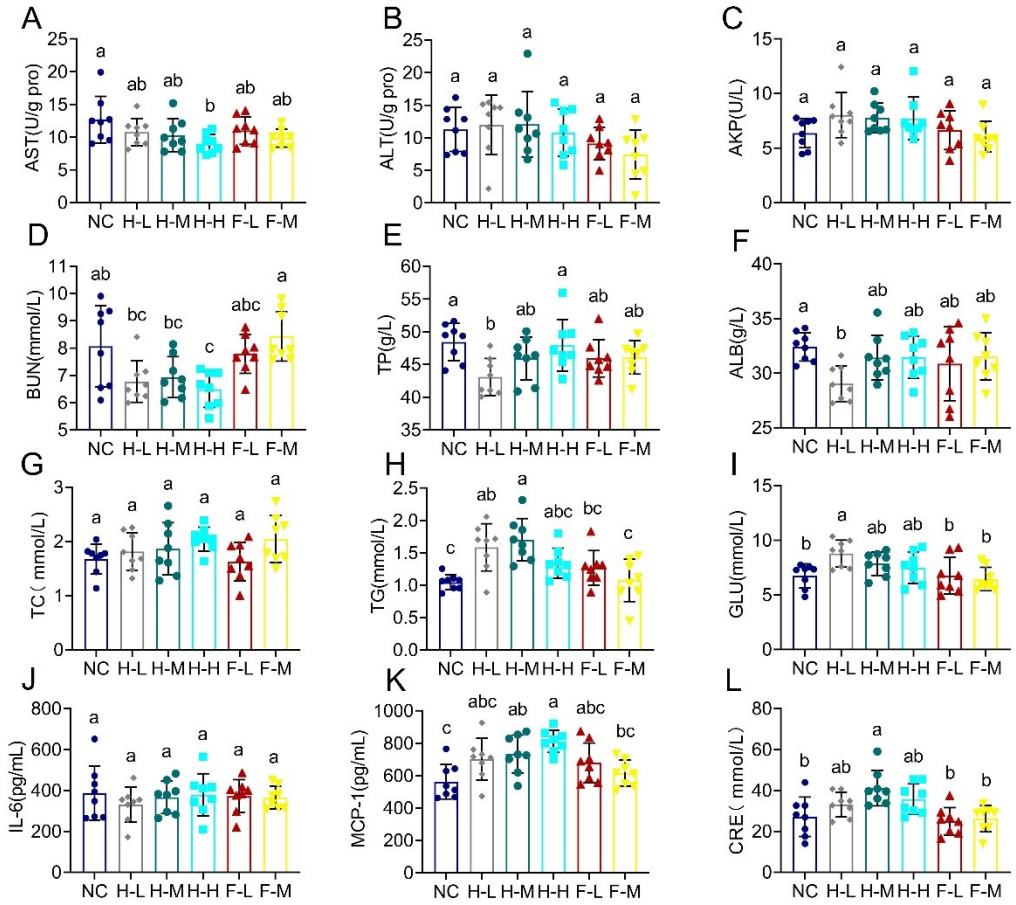

Fig. S14. The effect of oral administration of the EGCG-amyloid fibril hydrogels and the amyloid fibril solution samples on serum biochemical indexes of mice: aspartate aminotransferase (AST, A), alanine aminotransferase (ALT, B), alkaline phosphatase (AKP, C), blood urea nitrogen (BUN, D), total total protein (TP, E), albumin (ALB, F), cholesterol (TC, G), total glyceride (TG, H), glucose (GLU, I), interleukin-6(IL-6, J), monocyte chemoattractant protein 1 (MCP-1, K), creatinine (CRE, L) after the 9 week experiment. The amount of the hybrid hydrogels and the amyloid fibril solution sample was 200 μL each time. The EGCG dosage in the hydrogels were 100 mg/kg/day (H-L), 200 mg/kg/day (H-M) and 400 mg/kg/day (H-M). The dosage of the amyloid fibrils were 100 mg/kg/day (F-L) and 200 mg/kg/day (F-M).

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