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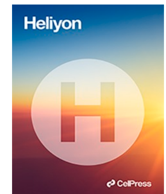

Arctium lappa L. roots inhibit the intestinal inflammation of dietary obese rats through TLR4/NF-κB pathway

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ABSTRACT

Long-term consumption of *Arctium lappa* L. roots can lead to weight loss. To explore the relationship between anti-obesity and anti-inflammation, the effects and mechanism of *A. lappa* L. root powder (ARP) on intestinal inflammation in obese rats were investigated. Dietary obese rats were successfully established by feeding a high-fat and high-sugar diet. The control group ($n = 6$) consumed a normal diet. The intestines were compared among the groups (each $n = 6$) with and without the administration of ARP (intragastric 7.5 g/kg·bw/d). Real-time quantitative reverse transcription-polymerase chain reaction and western blotting analysis revealed that ARP effectively inhibited the expression of pro-inflammatory and inflammatory cytokines in the colons of obese rats. These cytokines included interleukin (IL)-1β, IL-8, IL-6, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1. The inhibition rates for all these cytokines exceeded 88 %. Moreover, ARP demonstrated the ability to down-regulate key genes involved in Toll-like receptor 4 (TLR4) complexes, namely *Tlr4*, myeloid differentiation protein-2 (*Md2*), and myeloid differentiation factor 88 (*Myd88*), along with downstream signaling molecules such as tumor necrosis factor receptor associated factor 6 (TRAF6) and nuclear factor-κB (NF-κB), with inhibition rates over 81 %. Additionally, ARP was observed to inhibit protein levels of TLR4, NF-κB, IL-1β, and TNF-α in the colons of obese rats, with inhibition rates of 65.6 ± 10.9 %, 84.4 ± 19.9 %, 80.8 ± 14.4 %, and 68.4 ± 17.5 %, respectively. This study confirmed the effectiveness of ARP in inhibiting intestinal inflammation through the blockade of the TLR4/NF-κB signaling pathway. It also suggested that ARP holds potential in improving intestinal health in the context of obesity, implying its possible application in the prevention and treatment of obesity and related metabolic diseases.

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1. Introduction

Obesity is closely linked to a range of diseases, such as diabetes, dyslipidemia, hypertension, and several types of cancer [1]. It has become a major public health concern, affecting millions of people in both developing and developed countries. The World Health Organization has identified obesity as the leading chronic disease worldwide. Clinically, obesity is attributed to a complex interplay of factors, including diet, genetics, endocrine function, psychological well-being, social influences, and environmental factors [2]. Excessive body weight has been implicated in the development of metabolic syndrome, with obesity and insulin resistance playing critical roles in its pathogenesis. In obesity, visceral adipocytes tend to enlarge due to excessive accumulation of triglycerides. These enlarged adipocytes release lipid droplets into the surrounding area, triggering a local inflammatory response in macrophages. As a result, the phenotype of visceral adipocytes shifts from an anti-inflammatory state to a pro-inflammatory state. This leads to the production of inflammatory cytokines and chemokines by visceral adipocytes. Moreover, the number of macrophages in adipose tissue rapidly increases, establishing a vicious cycle of inflammation [3]. In recent years, accumulating evidence has highlighted the association between chronic low-grade inflammation in obesity and the gut. Dietary factors, the gut microbiota and its metabolites have been shown to influence gut immunity. Conversely, the intestinal immune system can impact intestinal permeability, immune cell transportation, and the supply of intestinal hormones [4]. Unhealthy lifestyle behaviors have been observed to lead to a significant increase in the pro-inflammatory intestinal macrophage subpopulation P2 in obese individuals. Notably, this increase in pro-inflammatory intestinal macrophages is observed throughout the gastrointestinal tract, including the stomach, duodenum, jejunum, ileum, and colon [5]. The down-regulation of NLRP6 (NACHT, LRR (leucine-rich repeat), and PYD (pyrin domain) domain-containing 6) and IL18 levels compromises the integrity of the intestinal barrier and initiates local inflammation. This inflammatory response subsequently affects dysfunctional adipocytes and promotes an uncontrolled cycle of inflammatory cascades, ultimately establishing a tumor-friendly microenvironment that favors the development of colon cancer [6]. Conversely, pharmacological interventions and weight loss may have beneficial effects on intestinal inflammation [5].

Intestinal tract, being the largest immune organ in the body, plays a critical role in immune regulation [7]. However, a high-fat and high-sugar (HF-HS) diet and certain unhealthy lifestyles, can disrupt the balance of bacterial populations in the gut [4]. Consumption of a HF-HS diet can trigger inflammation in the colon, leading to disrupted intestinal barrier function and increased permeability [8]. The imbalance in gut microbiota further contributes to this process, resulting in an elevation of lipopolysaccharide (LPS), a component of Gram-negative bacterial cell walls. This, in turn, promotes the growth of pathogenic bacteria and the release of LPS into the bloodstream, leading to an elevated systemic inflammatory response [9]. In a high-fat diet (HFD), the presence of free fatty acids and elevated levels of LPS can directly impact intestinal cells, triggering the release of excessive pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) [10,11]. Intestinal inflammation is associated with various diseases, including inflammatory bowel disease, irritable bowel syndrome, diverticulitis, infectious gastroenteritis, microscopic colitis, and so on. Furthermore, intestinal inflammation can contribute to a state of systemic chronic low-grade inflammation throughout the body, which is closely linked to obesity.

In the past few decades, numerous drugs have been developed to address obesity, including orlistat-based medications that act as lipase inhibitors to reduce fat absorption and aid weight loss. However, these drugs have been associated with significant side effects [12]. Consequently, researchers are increasingly focusing on exploring safe functional components derived from food, particularly edible or medicinal plants. Phytochemicals have gained attention as preferred anti-obesity compounds due to their safety, low toxicity, nutritional value, and pharmacological properties [13]. Many natural plant ingredients have been found to possess anti-obesity properties and promote overall health, making them valuable resources for food development.

Arctium lappa L., commonly known as burdock, is a traditional Chinese medicinal and edible plant that is known for its abundance of phytochemicals and polysaccharides, offering both nutritional and medicinal benefits. Burdock is primarily cultivated in Xuzhou, Jiangsu province, and Cangshan, Shandong province, in China. Fengxian, located in Xuzhou, is renowned as the "hometown of burdock". Burdock has gained popularity in Southeast Asian markets, particularly in Japan and Taiwan, owing to its distinctive aroma and high nutritional value. In recent decades, burdock has gained international recognition for its nutritional profile, biological activities, and positive impact on health [14]. Burdock contains a wide range of bioactive substances, including polysaccharide, polyphenols, flavonoids, and volatile oil, which contribute to its anti-inflammatory, antioxidant, antibacterial, antiviral, and other biological activities [15]. While traditionally used as an anti-inflammatory remedy, burdock has also shown potential anti-obesity effects in recent years. Research conducted by Ha et al. demonstrated that burdock root extract improved obesity in older women with metabolic syndrome [16]. Moreover, both aqueous and ethanol extracts of burdock have exhibited promising effects in preventing obesity by inhibiting white adipocyte differentiation and promoting brown adipocyte differentiation [17]. Furthermore, studies have demonstrated that the ethanolic extract of burdock root can modulate lipid metabolism levels and inhibit weight gain induced by a HF-HS diet in rats. This effect is believed to be mediated through the activation of the AMPK/ACC/CPT-1 pathway, which promotes hepatic fatty acid β-oxidation and improves hepatic steatosis [18]. Additionally, our unpublished data also suggests that *A. lappa* L. root powder (ARP) exhibits anti-obesity effect and improves lipid metabolism in obese rats fed a HF-HS diet. However, the specific underlying mechanism still remains unknown.

Obesity is characterized by chronic low-grade inflammation, and the link between gut inflammation and obesity is becoming increasingly evident. It is hypothesized that burdock may possess anti-obesity effects due to its anti-inflammatory properties, particularly in modulating intestinal inflammation. Therefore, the objective of this study was to investigate the effects of ARP on obese rats induced by a HF-HS diet, with a focus on elucidating the relationship between its anti-obesity and anti-inflammatory effects. The study involved examining changes in pro-inflammatory and inflammatory cytokines in the colons of obese rats. Additionally, the inflammatory regulatory signaling pathway was analyzed to explore the potential mechanisms by which burdock roots exert anti-

inflammatory effects in the intestinal tract of obese rats.

2. Materials and methods

2.1. Materials, chemical, and reagents

Arctium lappa L. (burdock, variety "Liuchuanlixiang") roots were sourced from the Burdock Germplasm Resource Base (Fengxian, China). Phosphate-buffered saline (PBS), polyvinylidene difluoride (PVDF) membranes, cell lysis buffer for Western blotting and immunoprecipitation, BCA (bicinchoninic acid) protein assay kit, Tris-glycine SDS (sodium dodecyl sulfate)-PAGE (polyacrylamide gel electrophoresis) running buffer (10 ×), Tris buffered saline with Tween 20 (TBST), Beyo enhanced chemiluminescence (ECL) Plus were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Defatted milk, SDS-PAGE preparation kit, EZ-buffers C Western transfer buffer were bought from Sangon Biotech Co., Ltd. (Shanghai, China). High purity total RNA extraction kit was purchased from Proteinssci Biotech Co., Ltd. (Shanghai, China). Goldband 3-color regular range protein marker (10–180 kDa) and SDS-PAGE protein loading buffer (5 ×) were obtained from Yeasen Biotechnology Co., Ltd. (Shanghai, China). ChamQ Universal SYBR qPCR Master Mix, HiScript III RT Super Mix for qPCR (+gDNA wiper), and 4×gDNA wiper Mix were bought from Vazyme Biotech Co., Ltd., (Nanjing, China). All these chemicals and reagents were of analytical grade.

2.2. Antibodies

The rabbit polyclonal primary antibody against TLR4 was obtained from Proteintech (Wuhan, China) and used at a dilution of 1:1000. The mouse monoclonal primary antibody against TNF-α was acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and used at a dilution of 1:200. The rabbit polyclonal primary antibody against the p105/p50 component of NF-κB was obtained from Ruiying Biological (Suzhou, China) and used at a dilution of 1:1000. Customized mouse polyclonal primary antibodies against IL-1β and β-Actin were acquired from Jiangsu Laisen Biotechnology Research Institute Co. LTD (Zhenjiang, China) and used at dilutions of 1:2000 and 1:10,000, respectively. The secondary antibodies, goat anti-rabbit/mouse horse radish peroxidase (HRP)-conjugated, were purchased from Abcam (Cambridge, United Kingdom) and used at a dilution of 1:1000.

2.3. Preparation of A. lappa L. Root powder

Fresh and pest-free burdock roots were carefully selected as the raw materials. After thorough cleaning with distilled water, the burdock roots were sliced into pieces with a thickness of 0.5–1 cm. These slices were then dried at a temperature of 60 °C for 4 h in a DGG-9140 constant temperature drying oven from Senxin Experimental Instrument Co., Ltd. (Shanghai, China) [19]. Once dried, the burdock root pieces were ground into a fine powder using an FW135 Chinese herbal medicine crusher from Tester Instrument Co., Ltd. (Tianjin, China). The powder was refined and sifted through a 60-size mesh sieve to ensure a consistent particle size. Finally, the powder marked with ARP was carefully sealed and store at −18 °C until further studies.

2.4. Animals and experimental design

A total of 30 male Sprague-Dawley (SD) rats, five weeks old, were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The rats were housed in standard laboratory conditions, with an ambient temperature maintained at 20–26 °C, humidity ranging from 40 % to 70 %, and a 12-h light-dark cycle. All animal experimental procedures were conducted in accordance with the guidelines set by the Research Animal Care and Use Committee at Nanjing Medical University, with the ethics approval number DWRL2020056. According to a previous study [18], out of the total 30 rats, six were randomly selected as the control group ($n = 6$). The control group was fed a normal diet (maintenance feed provided by Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd., Nanjing, China). To obtain obese rats, the remaining rats were fed a HF-HS diet (also provided by Jiangsu Xietong Pharmaceutical Bio-engineering Co., Ltd., Nanjing, China) for two weeks. The detail ingredient and nutrition composition of the rat diets can be found in Table S1. After 2 weeks of feeding, the change of body weight, feed utilization, and calories were statistically processed in both groups (Tables S2 and S3). Rats that did not develop obesity (increased weight compared with the control ≥20 %) were excluded from further studies. The obese rats were then randomly divided into two groups (each with $n = 6$), the HF-HS group and ARP treatment group. The ARP group received a daily intragastric administration of 7.5 g/kg·bw/d ARP, which was dissolved in distilled water and prepared at a concentration of 375 mg/mL. Both groups continued to be fed the HF-HS diet for an additional six weeks. At the end of the eighth week, all the rats were anesthetized, sacrificed, and their colons, representing the intestine tissues, were collected and stored at −80 °C for further studies.

2.5. Total RNA isolation

The colons were thawed and homogenized using a JY92-IIN ultrasonic cell crusher from Ningbo Xinzhi Biotechnology Co., Ltd. (Ningbo, China). Total RNA was isolated from the homogenized tissues using a high purity total RNA extraction kit following the protocol. The quality and quantity of the extracted RNA were assessed using a NanoDrop ND-2000 UV-VIS spectrophotometer from Thermo Fisher Scientific (Wilmington, Delaware, USA). The RNA samples with A_{260}/A_{280} and A_{260}/A_{230} ratios within the range of 1.8–2.0 were selected for further studies. The total RNA was dissolved in RNase and DNase free water and stored at −80 °C.

2.6. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

For each sample, a total of 1 μg of RNA was used for reverse transcription into cDNA using the HiScript III RT SuperMix for qPCR (+gDNA wiper) and 4×gDNA wiper Mix in a GE4852T gene amplifier from Hangzhou Bohang Technology Co., Ltd. (Hangzhou, China). The resulting cDNA was stored at −20 °C until further use. The cDNA was amplified using the ChamQ Universal SYBR qPCR Master Mixer according to the instruction. The PCR primers for the target genes and their sequences were listed in Table 1 [1,20,21]. Analysis of qRT-PCR was performed in a 96-well plate using an ABI 7300 StepOneTM Fast Real-Time PCR System from Applied Biosystems (Foster City, CA, USA). The amplification conditions were as follows: an initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. A melting curve analysis was conducted from 60 to 95 °C to ensure the amplification of a single product. To normalize the gene expression levels, the primer of *Gapdh* (glyceraldehyde 3-phosphate dehydrogenase) was amplified as a control in all the plates, serving as the housekeeper gene. The cycle threshold (Ct) values were used to calculate relative gene expression using the $2^{-\Delta\Delta Ct}$ method. All the data were expressed as fold increase over the corresponding control.

2.7. Western blotting analysis

The colons were lysed with cell lysis buffer for Western blotting and immunoprecipitation following the instructions. The protein concentration in the lysates was determined using the BCA protein assay kit. The denatured proteins were separated using SDS-PAGE and transferred onto a PVDF membrane. The membrane was then sealed with 5 % defatted milk dissolved in TBST and incubated at 25 °C for 1 h. Subsequently, the PVDF membrane was washed with TBST. To detect specific proteins, the membranes were probed with the primary antibodies against TLR4, NF-κB, IL-1β, and TNF-α, respectively, at 4 °C overnight. β-Actin was used as a loading control. After incubation, the membranes were washed three times with TBST. Next, the membranes were incubated with second antibodies at room temperature for 1 h. The protein signals on the membranes were visualized using ECL reagents and captured using an Image Quant™ LAS 4000 imaging system from GE Healthcare (Pittsburgh, PA, USA). All the data were expressed as fold changes relative to the control.

2.8. Statistical analysis

All the experiments were conducted in triplicate, and the data were presented as the mean ± standard error of the mean (SEM). The figures were generated using GraphPad Prism Version 8 software from GraphPad Software, Inc. (CA, USA). Statistical analysis was performed using one-way analysis of variance (ANOVA) to compare the mean of different groups followed by the Tukey test for post hoc analysis. In cases where applicable, repeated tests were conducted. Statistical significance was determined as $p < 0.05$.

Table 1
Primers for gene analysis of intestine in rats.

Target gene		Primer sequences (5′-3′)
Il1β (interleukin 1β)	Forward	GTGATGTTCCCATTAGACAGC
	Reverse	CTTTCATCACACAGGACAGG
Il8 (interleukin 8)	Forward	CATGGATCTGTCGTAGGGCT
	Reverse	CTGACCAACAGACCAGGGTT
Il6 (interleukin 6)	Forward	ATGAACTCCTTCTCCACAAGCGC
	Reverse	GAAGAGCCCTCAGGCTGGACTG
Tnfα (tumor necrosis factor-α)	Forward	TATGGCTCAGGGTCCAACTC
	Reverse	GGAAAGCCCATTTGAGTCCT
Mcp1 (monocyte chemoattractant protein-1)	Forward	TCAGCCAGATGCAGTTAACGC
	Reverse	TGATCCTCTTGTAGCTCTCCAGC
Icam1 (intercellular adhesion molecule-1)	Forward	CTGCAGAGCACAAACAGCAGAG
	Reverse	AAGGCCGCAGAGCAAAAGAAGC
Vcam1 (vascular cell adhesion molecule-1)	Forward	TAAGTTACACAGCAGTCAAATGGA
	Reverse	CACATACATAAATGCCGGAATCTT
Tlr4 (toll-like receptor 4)	Forward	TGAATCCCTGCATAGAGGTA
	Reverse	GACCGTTCTGTCATGGAAGG
Md2 (myeloid differentiation protein-2)	Forward	GTGGTTCTGCAACTCCTCCGATG
	Reverse	TGGCACAGAACTTCCTTACGCTTC
Myd88 (myeloid differentiation factor 88)	Forward	TACAAAGCAATGAAGAAGGA
	Reverse	TTGCATGAGGTAGTGGCACG
Traf6 (tumor necrosis factor receptor associated factor 6)	Forward	TCATTATGATCTGGACTGCCCAAC
	Reverse	TTATGAACAGCCTGGGCCAAC
Nfκb (nuclear factor-κB)	Forward	CAGCCTGGTGGGCAAGCACT
	Reverse	GAAGGATTTGGGGACTTT
Gapdh (glyceraldehyde 3-phosphate dehydrogenase)	Forward	CAGTGCCAGCCTCGTCTAT
	Reverse	AGGGGCCATCCACAGTCTTC

3. Results

3.1. Effects on the body weight and feed intake of obese rats

The body weights of rats in each group gradually increased with the increase of feeding days. After 6 weeks of feeding, the body weight of rats in the control increased 170.6 ± 19.2 g, while the body weight of rats in the HF-HS group increased 196.0 ± 24.4 g. Dietary intervention with ARP significantly reduced the body weight of rats, and the total weight gain of ARP rats (174.9 ± 31.8 g) gradually recovered to a similar level to that of the control (Table S4). There was no difference in weekly feed intake and total feed intake in the HF-HS group compared with the control, whereas weekly feed intake and total feed intake in the ARP group were lower than those in the HF-HS group (Table S5). The total calorie intake of the rats in the HF-HS group were significantly higher than those of the control or ARP group ($p < 0.05$), while the total calorie intake of the rats in the ARP group was higher than that of the control (Table S6). Therefore, the ARP group reached the similar weight gain with a higher total calorie intake than the control. The lower feed intake might attribute to the anti-obesity activity of ARP. Subsequent autopsy of the rats showed that dietary intervention with ARP significantly decreased the body fat, pararenal fat, as well as blood lipids, including serum cholesterol and triglyceride (Table S7), further confirming the anti-obesity activity of ARP. This is due to the presence of a large amount of dormant brown adipose tissue around both kidneys, which was activated after ARP administration, and the brown adipose tissue has the effect of inhibiting lipid deposition.

3.2. Effects on the mRNA levels of pro-inflammatory cytokines in the intestinal tract of obese rats

Interleukin-1β is a key cytokine that is secreted during intestinal inflammation [21]. It plays a critical role in the development of intestinal inflammation and activates NF-κB, which is a crucial marker of cellular inflammation and contributes to inflammatory responses [22,23]. IL-1β holds particular significance among cytokines in the context of intestinal inflammation, and clinical studies have reported the elevated levels of IL-1β secreted by colonic lamina propria monocytes in patients with active inflammatory bowel disease [24,25]. Following intervention with a HF-HS diet, the mRNA expression of *Il1β* in the colons significantly increased when compared to the control (3.87 ± 0.47-fold change, $p < 0.0001$). However, treatment with ARP resulted in a decrease in *Il1β* mRNA

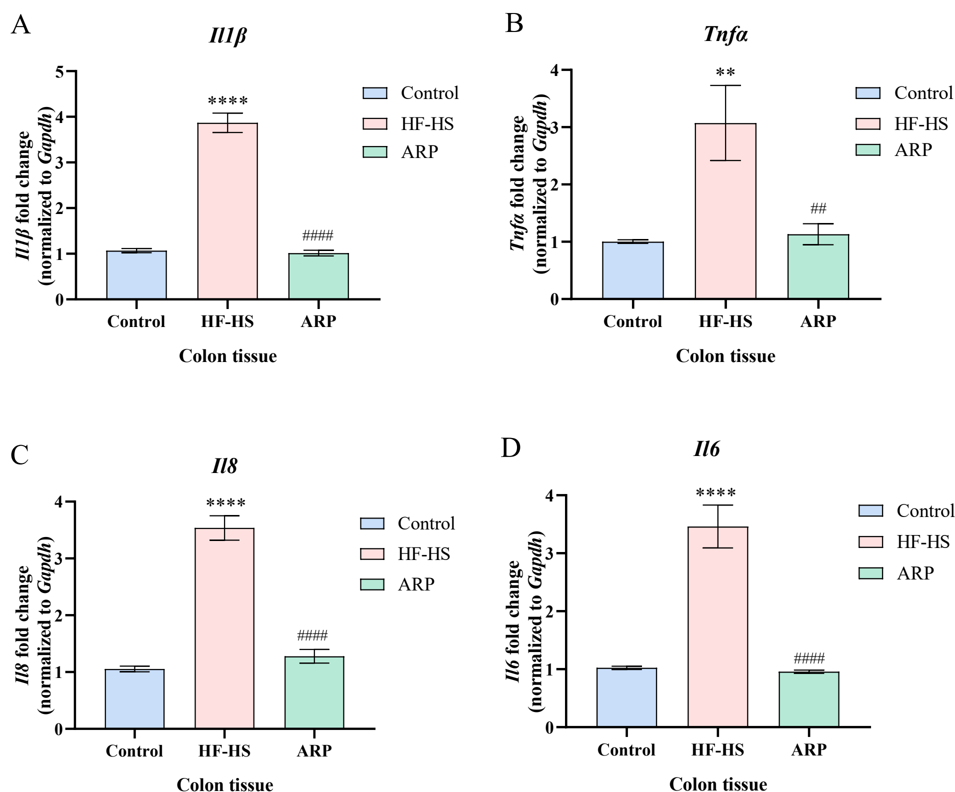

Fig. 1. The effects of *Arctium lappa* L. root powder (ARP) on mRNA expression of pro-inflammatory cytokines in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. (A) Interleukin-1β (*Il1β*), (B) tumor necrosis factor-α (*Tnfα*), (C) *Il8*, and (D) *Il6*. All data are represented as the mean ± standard error of the mean (SEM, n = 6). ** and **** indicate $p < 0.01$ and $p < 0.0001$ compared with the control, while ## and #### indicate $p < 0.01$ and $p < 0.0001$ compared with the HF-HS group, respectively.

expression to the normal level. The fold change in ARP-fed rat colons was only 1.02 ± 0.17-fold change ($p > 0.05$) compared to the control group (Fig. 1A).

Tumor necrosis factor (TNF)-α is a critical pro-inflammatory cytokine involved in the inflammatory process. It stimulates the

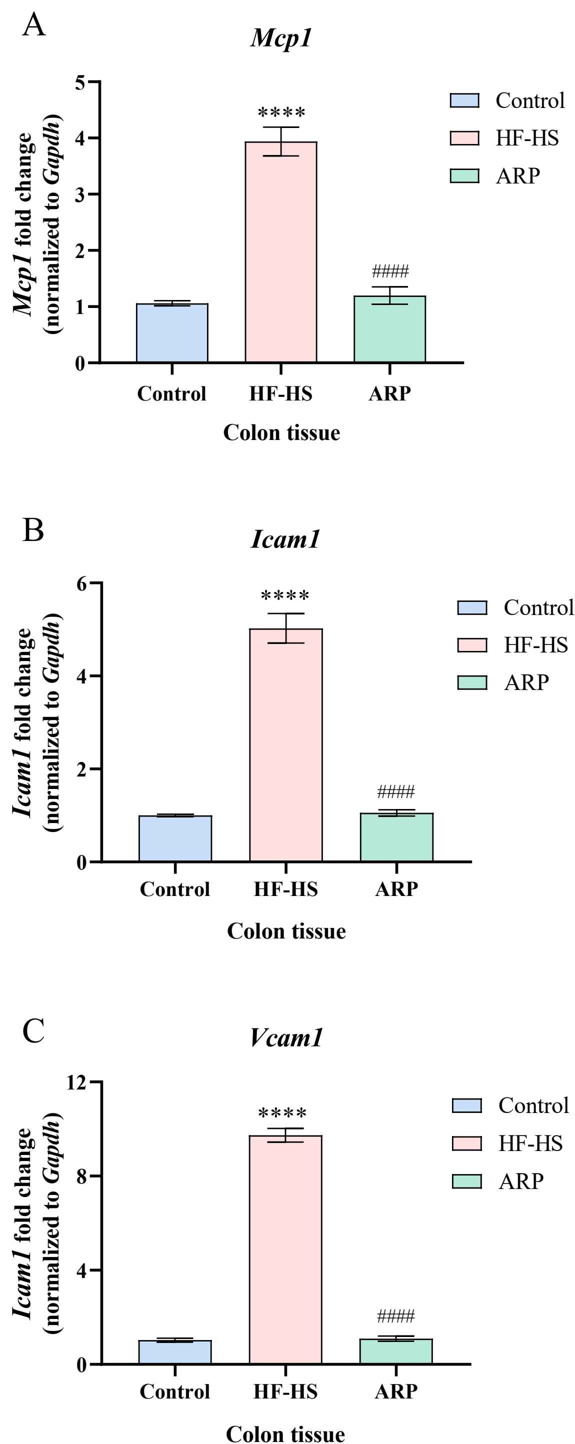

Fig. 2. The effects of *Arctium lappa* L. root powder (ARP) on mRNA expression of inflammatory cytokines in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. (A) monocyte chemoattractant protein-1 (*Mcp1*), (B) intercellular adhesion molecule-1 (*Icam1*), and (C) vascular cell adhesion molecule-1 (*Vcam1*). All data are represented as the mean ± standard error of the mean (SEM, n = 6). **** indicates $p < 0.0001$ compared with the control, while #### indicates $p < 0.0001$ compared with the HF-HS group, respectively.

secretion of various pro-inflammatory mediators and induces the expression of other inflammatory molecules [26]. Plasma levels of TNF-α have been observed to be significantly elevated in obese adults compared to individuals with normal-weight [27]. In this study, the mRNA expression of *Tnfα* in the colons of rats fed with ARP (1.13 ± 0.41-fold change, $p > 0.05$ *vs* the control and $p < 0.01$ *vs* the HF-HS group) was significantly decreased in comparison with the HF-HS group (3.07 ± 1.46-fold change, $p < 0.01$ *vs* the control). This

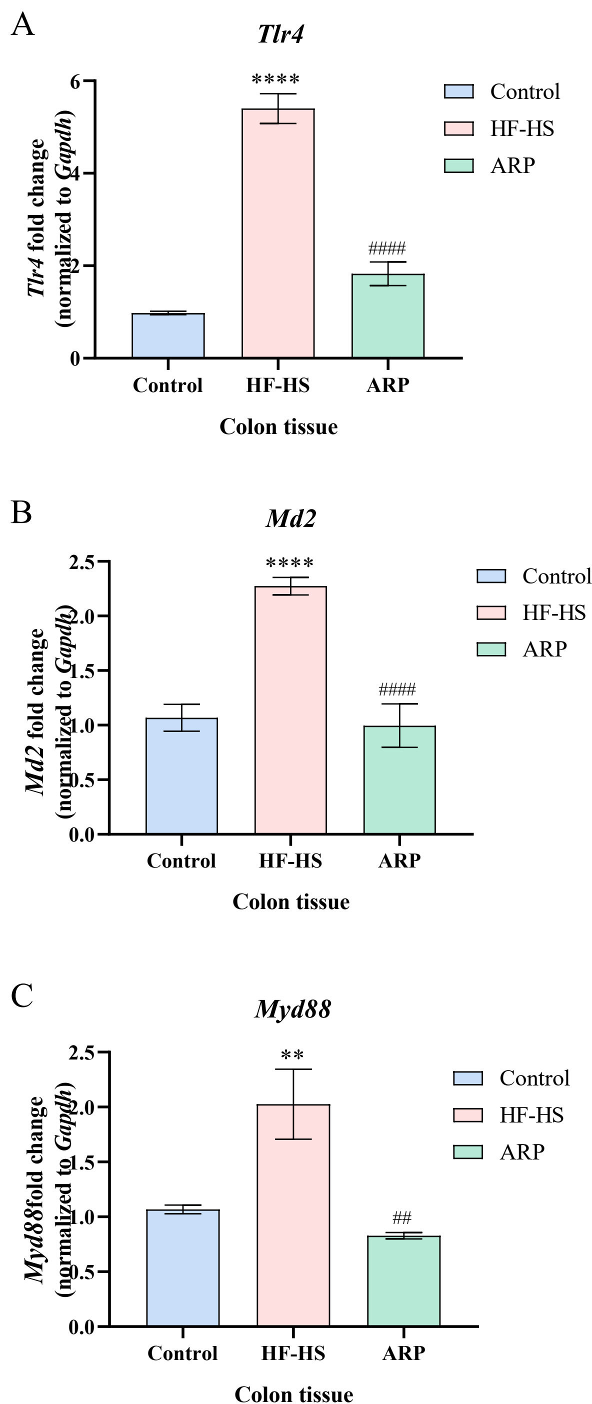

Fig. 3. The effects of *Arctium lappa* L. root powder (ARP) on key gene mRNA expression of toll-like receptor 4 (*Tlr4*) complexes in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. (A) *Tlr4*, (B) myeloid differentiation protein-2 (*Md2*), and (C) myeloid differentiation factor 88 (*Myd88*). All data are represented as the mean ± standard error of the mean (SEM, n = 6). ** and **** indicate $p < 0.01$ and $p < 0.0001$ compared with the control, while ## and #### indicate $p < 0.01$ and $p < 0.0001$ compared with the HF-HS group, respectively.

suggests that ARP had the ability to inhibit the overexpression of pro-inflammatory factors in the intestinal tract, specifically TNF-α (Fig. 1B).

Interleukin-8 is a cytokine that is produced by macrophages and other cells during the early stages of infection [28]. It plays a critical role in recruiting innate and adaptive immune cells to the site of infection [29]. In the intestinal epithelium, IL-8 is one of the earliest chemokines produced by epithelial cells. It is released to initiate an acute inflammatory response for combatting pathogenic infections [28–30]. In the HF-HS group, the mRNA expression of *Il8* was approximately 3.54 ± 0.48-fold change, which was significantly higher than that in the control and the ARP group ($p < 0.0001$). However, in the rats fed with ARP, the mRNA expression of *Il8* in the colons (1.28 ± 0.27-fold change) was significantly decreased, showing a comparable level to that of the control group (Fig. 1C).

Interleukin-6 is a pro-inflammatory cytokine that is secreted by adipocytes, macrophages, and T cells. It plays a role in the acute phase of inflammation and aids the host in defending against emergent stress, such as infection or tissue damage, by activating an immune response [31]. IL-6 has also been implicated in the development of colon cancer and colitis-related cancers [32,33]. In this study, ARP was found to down-regulate the increased expression of *Il6* mRNA in the rat intestine induced by a HF-HS diet. The results showed that the relative expression of *Il6* mRNA in the colons of rats fed with ARP (0.96 ± 0.06-fold change, $p > 0.05$) was similar to that of the control group. However, it was significantly lower than the HF-HS group (3.46 ± 0.83-fold change, $p < 0.0001$, Fig. 1D).

3.3. Effects on the mRNA levels of inflammatory cytokines in the intestinal tract of obese rats

Monocyte chemoattractant protein-1 (MCP-1) is an important inflammatory factor involved in chronic inflammation associated with obesity. It plays a significant role in obesity-related metabolic disorders [34]. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are inflammatory molecules predominantly secreted by endothelial cells during

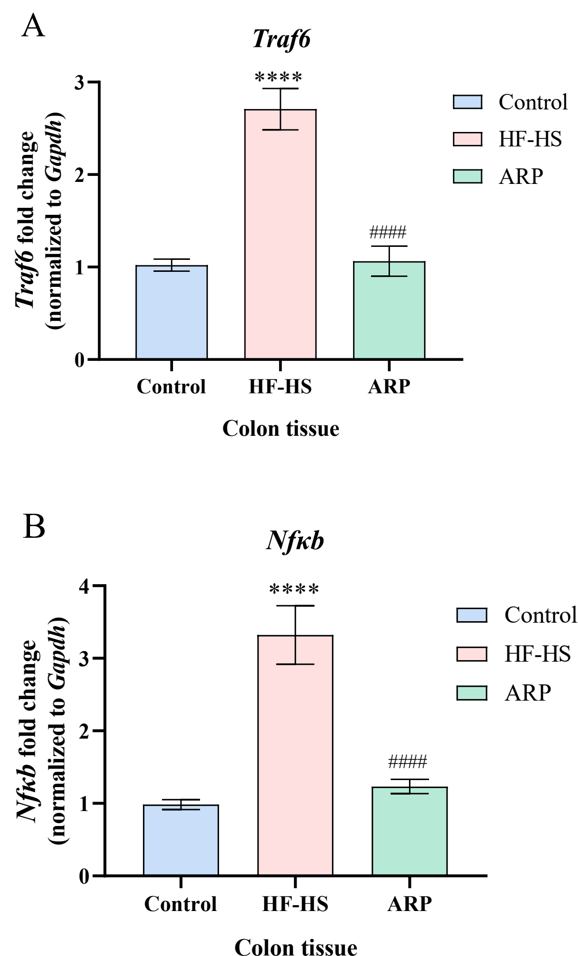

Fig. 4. The effects of *Arctium lappa* L. root powder (ARP) on key gene mRNA expression of toll-like receptor 4 (*Tlr4*) downstream signals in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. (A) tumor necrosis factor receptor associated factor 6 (*Traf6*) and (B) nuclear factor-κB (*Nfκb*). All data are represented as the mean ± standard error of the mean (SEM, n = 6). **** indicates $p < 0.0001$ compared with the control, while #### indicates $p < 0.0001$ compared with the HF-HS group, respectively.

inflammation. They serve as indicators of endothelial injury [35]. These inflammatory cytokines are primarily regulated by NF-κB signaling pathway and contribute to inflammatory responses [36,37]. In order to further explore the inhibitory effect of ARP on intestinal inflammation in rats fed a HF-HS diet, we selected downstream inflammatory factors of NF-κB as indicators to determine

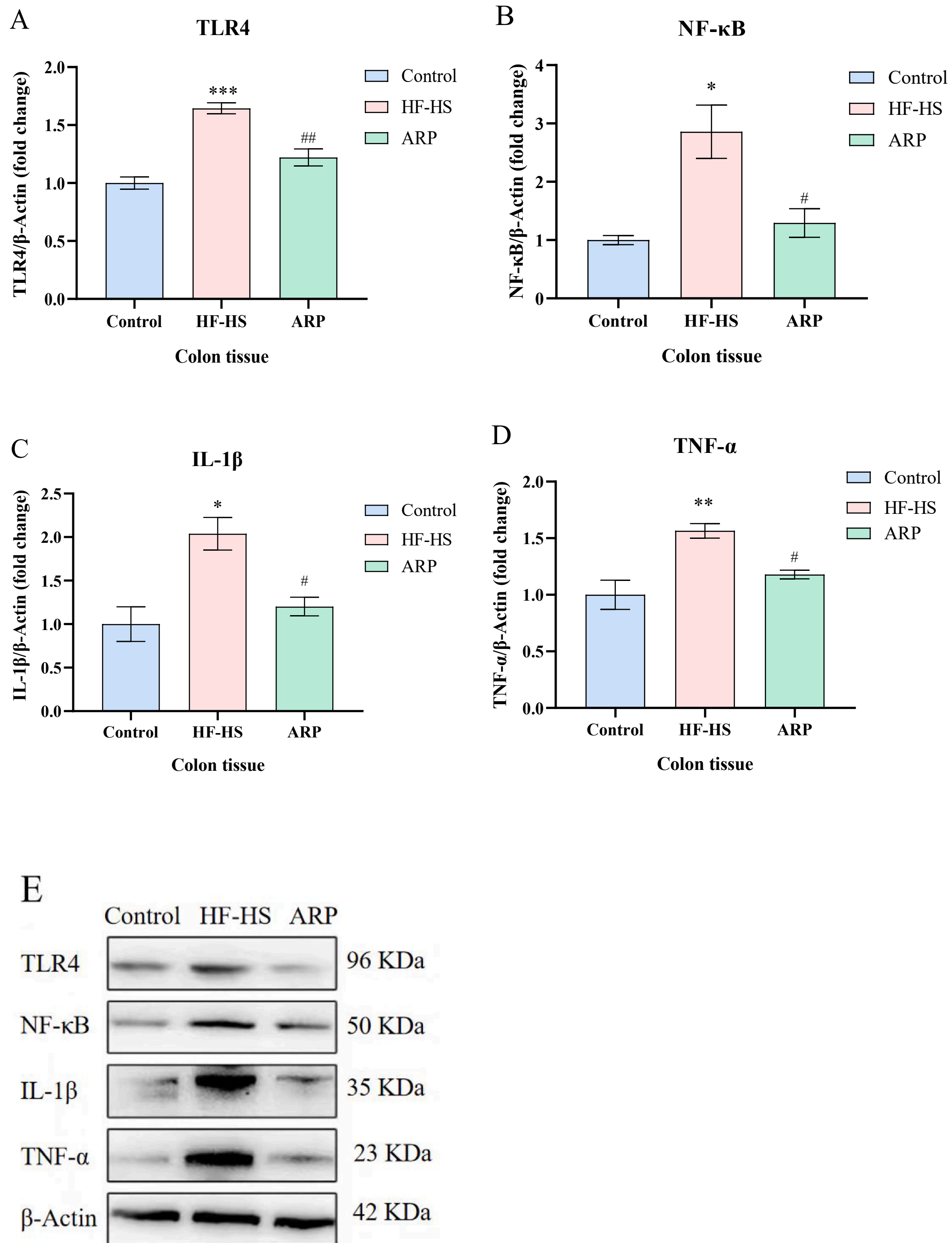

Fig. 5. The effects of *Arctium lappa* L. root powder (ARP) on relative protein levels of pro-inflammatory cytokines by blocking toll-like receptor 4 (TLR4) signaling pathway in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. (A) The representative Western blot bands of TLR4, nuclear factor-κB (NF-κB), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) are shown, (B) TLR4, (C) NF-κB, (D) Il-1β, and (E) TNF-α fold change. All data are represented as the mean ± standard error of the mean (SEM, n = 6). *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ compared with the control, while #, ##, and ### indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$ compared with the HF-HS group, respectively.

whether ARP could protect intestinal tissue and reduce inflammatory damage by regulating the mRNA expression of *Mcp1*, *Icam1* and *Vcam1*.

The mRNA expression level of *Mcp1* in the HF-HS group was significantly higher than that in the control, with a fold change of approximately 3.94 ± 0.57 ($p < 0.0001$, Fig. 2A). However, in the ARP group, the mRNA expression level of *Mcp1* was significantly lower than that in the HF-HS group and was not significantly different from that of the control ($p > 0.05$). Similarly, the mRNA expression of *Icam1* and *Vcam1* in HF-HS group were approximately 5.02 ± 0.7-fold change and 9.73 ± 0.65-fold change, respectively (Fig. 2B and C). These values were significantly higher than those in the control and the ARP group ($p < 0.0001$). The mRNA expression levels of *Icam1* and *Vcam1* in the ARP group were similar close to those in the control. These results indicate that ARP effectively inhibits the release of inflammatory cytokines in the intestinal tract induced by a HF-HS diet.

3.4. Effects on mRNA levels of key genes on TLR4/NF-κB signaling pathways in the intestinal tract of obese rats

Toll-like receptor 4 (TLR4) is an important innate immune recognition molecule that acts as a transmembrane glycoprotein, responsible for transmitting signals across cell membranes. It plays a critical role in promoting the synthesis and release of pro-inflammatory and inflammatory cytokines [38]. In this study, we focused on several key genes involved in the TLR4/NF-κB signaling pathway. These included the TLR4 complexes (*Tlr4*, *Md2*, and *Myd88*, as shown in Fig. 3A–C) and downstream signaling molecules (*Traf6* and *Nfκb*, as shown in Fig. 4A and B). The mRNA levels of these genes were investigated using qRT-PCR. The results showed that the mRNA expressions of *Tlr4*, *Md2*, and *Myd88*, *Traf6*, and *Nfκb* in the colons of rats fed with a HF-HS diet were significantly increased compared to those in the control ($p < 0.01$). The mRNA expression levels were 5.40 ± 0.72, 2.28 ± 0.18, 2.03 ± 0.71, 2.71 ± 0.50, 3.32 ± 0.90-fold change, respectively. However, the mRNA expression of these genes was inhibited by ARP treatment in the colons of rats ($p < 0.01$). There was no significant difference in mRNA expression between the ARP group and the control ($p > 0.05$). Based on these findings, it can be inferred that ARP may exert its inhibitory effects on intestinal inflammation induced by a HF-HS diet, possibly through the modulation of TLR4/NF-κB signaling pathway.

3.5. Effects on protein levels of related signaling pathway and downstream factors in the intestinal tract of obese rats

The effects of ARP on the protein levels of the TLR4/NF-κB signaling pathway and downstream pro-inflammatory/inflammatory cytokines in the intestinal tract of obese rats were also examined. The results showed that the transcription levels of key genes in the TLR4/NF-κB signaling pathway and the protein expressions of TLR4 and NF-κB were significantly upregulated in the intestinal tissues of rats fed a HF-HS diet compared to the control (1.64 ± 0.07-fold change, $p < 0.001$; and 2.86 ± 0.79-fold change, $p < 0.05$, respectively). However, treatment with ARP effectively downregulated the protein expressions of TLR4 and NF-κB (1.22 ± 0.10-fold change, $p < 0.01$; and 1.29 ± 0.42-fold change, $p < 0.05$, respectively; Fig. 5A and B). Furthermore, the protein expressions of pro-inflammatory cytokines IL-1β and TNF-α were significantly increased in the colons of rats fed a HF-HS diet. However, ARP treatment inhibited this increase, resulting in significantly lower protein levels of IL-1β and TNF-α (1.20 ± 0.15 and 1.18 ± 0.05-fold change, respectively) compared to the HF-HS group (2.04 ± 0.27 and 1.57 ± 0.10-fold change, respectively, $p < 0.05$, Fig. 5C and D). Importantly, there were no significant differences in the protein expressions of TLR4, NF-κB, IL-1β, and TNF-α between the control and ARP group ($p > 0.05$). These findings suggest that ARP can effectively inhibit the upregulation of pro-inflammatory factors induced by a HF-HS diet in the intestinal tissues by downregulating the TLR4/NF-κB signaling pathway at both mRNA and protein levels (Fig. 5E).

4. Discussion

Obesity is primarily attributed to a chronic imbalance between energy intake from dietary sources and energy expenditure through physical activity [39]. Chronic low-grade inflammation is a characteristic feature of obesity. In the context of a HFD, the presence of free fatty acids and/or lipopolysaccharide (LPS) can directly stimulate intestinal cells, resulting in the excessive release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [10,11]. These inflammatory mediators contribute to the development of a systemic, chronic, low-grade inflammatory state throughout the body. The intestinal immune system plays a significant role in regulating glucose homeostasis and obesity-related insulin resistance [40]. Consumption of high-sugar diets [41] and high-fat diets [42] can increase intestinal permeability, disrupt the integrity of intestinal epithelium, and result in inflammatory changes and the release of pro-inflammatory mediators. In the context of obesity, dietary factors, intestinal flora, and their metabolites can impact intestinal immunity. A study conducted on obesity-prone (DIO-P) and obesity-resistant (DIO-R) rats investigated the effects of a HFD on the microbial community. The results revealed that the HFD induced alterations in the microbial composition of both DIO-P and DIO-R rats. However, only the DIO-P rats exhibited inflammation in the small intestine, manifested by reduced levels of intestinal alkaline phosphatase (IAP), increased TLR4 signaling, enhanced intestinal permeability, and elevated plasma LPS levels. The study provides robust evidence linking intestinal inflammation to obesity, potentially attributed to decreased IAP levels and specific changes in the gut microbiota composition alterations [43]. Therefore, in this study, we established an obesity model in SD rats with the goal of exploring the potential relationship between anti-obesity and intestinal inflammation inhibitory effects of ARP. The significant difference in body weight gain between the control and the HF-HS groups indicated that the rat obesity model was successfully established ($P < 0.05$, Tables S2 and S3).

Nutritional interventions have become essential in the study of obesity control, with a focus on regulating hormones and their downstream effectors in adipose tissue, liver, and the gut. Burdock, known for its richness in nutrients and bioactive ingredients, has been extensively researched for its potential health benefits [44,45]. In our previous study (unpublished data), we discovered that

burdock root powder enhanced the metabolism of fatty acids in the liver, stimulated lipolysis, and reduced fat accumulation in obese rats. In this study, dietary intervention with ARP exhibited significant anti-obesity activity. The total weight gains of ARP rats gradually recovered to a similar level to that of control rats although ARP rats had a higher total calorie intake than the control group, which may attribute to its lower feed intake than the control and the HF-HS group ($P < 0.05$, Tables S4–S6). The significant decrease of body fat weight, fat rate, pararenal fat weight, and blood lipids (including cholesterol and triglyceride) in the ARP intervention group reflected the anti-obesity advantage of ARP, since the dyslipidemia induced by the HF-HS diet was alleviated by ALP dietary intervention (Table S7). In addition, obesity-induced intestinal inflammation was also significantly reduced, as was the mRNA expression of *Il1β*, *Tnfα*, *Il8*, and *Il6* in the rat colon. These results suggest that ARP significantly reduced body weight and suppressed the overexpression of intestinal pro-inflammatory factors in rats.

The TLR4 signaling pathway is widely recognized as a significant contributor to obesity-induced inflammation [38]. It is well-established that TLR4 is activated by LPS and plays a crucial role in mediating the interaction between inflammatory and metabolic signals [37,46]. The TLR4 is an extracellular receptor that plays a critical role in recognizing antigenic information. Upon activation by LPS or fatty acids, TLR4 forms a complex with myeloid differential protein-2 (MD-2). This complex transmits signals into the cell, triggering an inflammatory response. Additionally, MyD88 binds to the TLR4 complexes and transmits signals to downstream molecules, including interleukin-1 receptor-associated kinase 4 (IRAK4). This activation leads to subsequent signaling cascades. Tumor necrosis factor receptor associated factor 6 (TRAF6) is activated by IRAK4, resulting in the activation of NF-κB and subsequent excessive production of various pro-inflammatory mediators such as IL-1β, IL-6, IL-8, and TNF-α [47]. Nuclear factor-κB (NF-κB) is a vital transcription factor that plays a pivotal role in classical signal transduction pathways and gene regulation. It is closely associated with a variety of diseases, particularly inflammatory conditions. Activation of NF-κB initiates the transcription and expression of pro-inflammatory genes, leading to the excessive accumulation of various mediators, including pro-inflammatory and inflammatory cytokines [48]. The secretion of these cytokines can further stimulate the activation of inflammatory cells, leading to an inflammatory

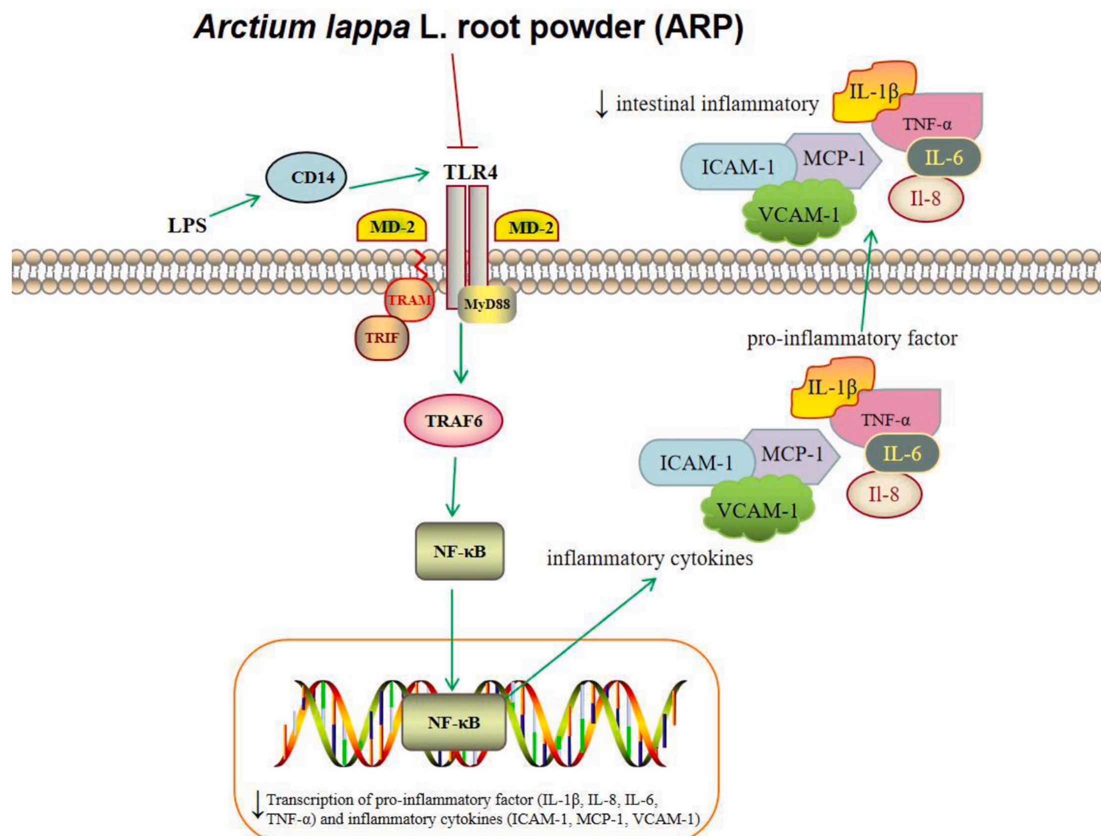

Fig. 6. Schematic of *Arctium lappa* L. root powder (ARP) inhibiting intestinal inflammation *in vivo* by TLR4/NF-κB signaling cascades. The block of TLR4/NF-κB signal transduction through the down-regulation on key genes of toll-like receptor 4 (TLR4) complexes (TLR4, MD-2, and MyD88) and downstream signals (TRAF6 and NF-κB) leads to lower pro-inflammatory and inflammatory cytokines (IL-1β, IL-8, IL-6, TNF-α, MCP-1, ICAM-1, and VCAM-1) in the colons from obese rats induced by high-sugar and high-fat (HF-HS) diet. MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation factor 88; TRAF6, tumor necrosis factor receptor associated factor 6; NF-κB, nuclear factor-κB; IL-1β, interleukin-1β; IL-8, interleukin-8; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1.

overreaction and damage to host tissues and organs. Research has indicated that intestinal epithelial TLR4 plays a protective role in preventing metabolic syndrome by regulating the interactions between microorganisms and intestinal epithelial cells in mice [49]. In addition, TLR4 plays an important role in fat-induced visceral inflammation in rats [50]. Moreover, amitriptyline, an antidepressant drug known for its effects on weight modulation [51,52], has been found to inhibit the release of pro-inflammatory cytokines in rats with colitis through the TLR4/NF-κB signaling pathway [53]. Furthermore, loss-of-function mutations in TLR4 have been demonstrated to protect mice against diet-induced obesity [54]. In this study, it was confirmed that a HF-HS diet induces gut inflammation, leading to the production of pro-inflammatory and inflammatory cytokines. The TLR4/NF-κB signaling pathway was found to play a crucial role in the inhibitory effects of burdock roots on intestinal inflammation in obese rats. Fig. 6 illustrates the schematic representation of the signaling cascade of ARP *in vivo*. *Arctium lappa* L. root powder was shown to inhibit the signal transduction by down-regulating TLR4 complexes (TLR4, MD-2, and MyD88) and key downstream signals (TRAF6 and NF-κB), resulting in a reduction in pro-inflammatory and inflammatory cytokines, including IL-1β, IL-8, IL-6, TNF-α, MCP-1, ICAM-1, and VCAM-1 in the colons of obese rats induced by a HF-HS diet.

These findings suggested that the improvement of obesity-induced inflammation by burdock may be linked to the modulation of the TLR4/NF-κB signaling pathway. However, the whole powder of burdock roots was investigated in this study, which contained various bioactive constituents. We need to further clarify which constituents have the ability to modulate the gut and exert anti-obesity effects. Burdock roots are known to be rich in polysaccharides [55] and polyphenols [15]. Previous studies have demonstrated that plant polysaccharides and polyphenols can regulate glucose and lipid metabolism, effectively improving obesity and inflammation through the TLR4/NF-κB signaling pathway [56,57,58]. Further studies are necessary to identify the specific polysaccharides and polyphenols present in burdock roots responsible for its anti-inflammatory effects. In addition, more dose of ARP should be measured to make sure if ARP will act in a dose-dependent manner or dose-independent manner, and to explore their intestinal metabolites, as well as to assess the safety of burdocks.

5. Conclusion

The results of this study demonstrated that a HF-HS diet induced the production of pro-inflammatory and inflammatory cytokines in intestinal tissues. *Arctium lappa* L. root powder effectively inhibited intestinal inflammation in obese rats by regulating immune and inflammatory responses through the TLR4/NF-κB signaling pathway. This inhibition leads to a reduction in the release of pro-inflammatory cytokines and inflammatory factors. These findings provide a scientific basis for the development of functional foods using burdock roots to enhance intestinal health in individuals with obesity and related metabolic disorders diseases. The study also has significant implications for understanding the physiological functions and mechanisms of burdock and its potential applications. Future studies will focus on identifying the specific constituents in burdock roots that contribute to the anti-inflammatory and anti-obesity effects, as well as exploring the correlation between these constituents and genes involved in gut barrier function, inflammation, and lipid transportation.

Data availability statement

Data will be made available on request.

Ethics declarations

This study was reviewed and approved by the Research Animal Care and Use Committee at Nanjing Medical University, with the approval number: DWRL2020056.

CRediT authorship contribution statement

Feng Zeng: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Data curation. **Ying Li:** Validation, Project administration, Funding acquisition, Conceptualization. **Xiaoxiao Zhang:** Writing – original draft, Methodology, Data curation. **Jin Feng:** Software, Project administration, Conceptualization. **Wen Gu:** Project administration, Conceptualization. **Li Shen:** Writing – review & editing, Validation, Software, Project administration, Conceptualization. **Wuyang Huang:** Writing – review & editing, Validation, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21562.

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