

Dendrobium officinale Six nostrum promotes excretion of intestinal uric acid in hyperuricemia rat through inhibiting LPS/TLR4/NF- κ B signaling pathway

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Research

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***Dendrobium officinale* Six nostrum promotes excretion of
intestinal uric acid in hyperuricemia rat through inhibiting
LPS/TLR4/NF- κ B signaling pathway**

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Abstract

Background: To evaluate the effect of *Dendrobium officinale* Six nostrum (DOS) on promoting the intestinal excretion of uric acid (UA) in the rat model of hyperuricemia (HUA), and explored its possible mechanisms of action.

Methods: In this study, HUA was induced in rat by administration of lipid emulsion (LE) for 6 weeks, meanwhile, the rat was orally administered with DOS for 6 weeks. Then the level of serum uric acid (SUA) and fecal uric acid (FUA) were measured by automatic biochemical analyzer. Lipopolysaccharide (LPS) in hepatic portal vein blood was detected by ELISA kit. The intestinal protein levels of ATP-binding cassette superfamily G member 2 (ABCG2) and glucose transporter 9 (GLUT9) were measured by Western blot assay. Toll-like receptor 4 (TLR4) and nuclear factor kappa-B (NF- κ B) were determined by immunohistochemistry (IHC). Hematoxylin and eosin (H&E) staining was used to assess intestinal histological changes. Meanwhile, the main compositions of DOS were identified and determined by the High Performance Liquid Chromatography (HPLC).

Results: According to HPLC analysis, acteoside and astilbin were identified as the main chemical components of DOS. Our results indicated that DOS significantly reduced SUA level and increased FUA level in the HUA rat induced by oral administration of LE for 6 weeks. IHC and Western blot showed that DOS could significantly increase protein level of ABCG2 and reduce protein level of GLUT9 in the intestine. It remarkably reduced the content of LPS in hepatic portal vein blood and decrease protein levels of TLR4 and NF- κ B in the intestine. In addition, DOS could improve the histopathological changes of intestine through increasing the number of intestinal gland goblet cells, and recovering the complete and neatly-arranged structure of small intestinal epithelial cells.

Conclusions: DOS has the effect of treating hyperuricemia, the molecular mechanism may be associated with up-regulating ABCG2 protein level, and down-regulating protein level of GLUT9 in the intestine to promote the intestinal excretion of UA, and then decreasing the SUA level. In addition, DOS can reduce the damage of inflammatory response to the intestine, improve the histopathological changes of intestine in HUA rat through inhibiting LPS/TLR4/NF- κ B inflammatory pathway.

Keywords: *Dendrobium officinale*; hyperuricemia; uric acid; intestine; uric acid transporter.

Background

UA is the final product of human purine metabolism, SUA levels are determined by the balance between UA production and UA excretion rates. UA is mainly produced in the liver^[1]. Approximately 2/3 of daily urate is excreted by the kidney, and the other 1/3 of uric acid is secreted by the intestine^[2, 3]. Clinically, excessive production and/or reduced excretion of UA may lead to HUA^[4-6]. In recent years, many studies have suggested that HUA is closely associated with many diseases, including gout^[7-9], hypertension^[10-12] and hypertriglyceridemia et al^[13, 14]. It is reported that in the past few decades, the worldwide prevalence rate of HUA reported to be ranging from 2.6% to 36% in different populations^[15]. With the rapid economic development, the lifestyle of the Chinese has changed greatly, the prevalence rate of HUA in mainland China was 13.3%^[16] (19.4% in men and 7.9% in women).

At present, there is no ideal drug for the treatment of HUA. Most of the drugs selected in the clinical treatment are urate transporter selective inhibitors and xanthine oxidase (XOD) inhibitors. However, long-term use of drugs to inhibit the production or promote the excretion of uric acid, such as Febuxostat^[17] (XOD inhibitors) and benzbromarone^[18] (UA excretor) may cause a series of adverse reactions, including severe hypersensitivity^[19], and serious liver and kidney damage^[20, 21].

In recent years, the role of the intestine^[20, 21] in maintaining the homeostasis of UA level in the body has become the focus of attention. It has been found that the reduction of intestinal excretion of UA^[23, 24] is an important cause of HUA. Intestinal UA excretion is regulated by urate transporters^[25], such as ABCG2, GLUT9, MRP4 and NPT5. Many studies have demonstrated that ABCG2^[26] is expressed in the gastrointestinal tract at high levels, especially in the intestine, which plays an important role in excreting UA from intestine into feces. GLUT9^[27] is the only member of the GLUT family that involve in the reabsorption of UA in the body Reducing UA levels by improving intestinal UA excretion and inhibiting the reabsorption of UA in the intestine may be an effective prevention and treatment for HUA and its complications.

Traditional Chinese medicine is an important research field in the treatment of HUA, which has the potential to be ideal drug for the treatment of HUA. *Dendrobium*

officinale^[27], one of the most famous species of *Dendrobium*, has long been regarded as precious herbs and healthy foods applied in TCM and folk. Based on the classic Simiao Wan prescription, our laboratory has made the *Dendrobium officinale* six nostrum (DOS). The whole prescription was composed of six traditional Chinese herbs such as *Dendrobium officinale* and *Atractylodes macrocephalae* rhizoma etc. In our previous study, the rat model of HUA was established by feeding on high-fat and high purine diet and orally administered with potassium oxonate. The SUA level in the rat model of HUA significantly decreased after oral administration of DOS, and DOS can improve the pathological changes of intestine, which indicated that DOS had the effect of reducing UA level and protecting the intestinal tract. In this study, we have proved that DOS^[29] had the lowering effect on UA level in rat model of HUA through the kidney, therefore, further explored whether DOS can reduce UA level via the intestine.

This study aimed to investigate the effect of DOS on intestinal excretion of UA in the HUA rat induced by oral administration of lipid emulsion (LE) for 6 weeks according to our previous reports^[29], and explore the molecular mechanism of its action by LPS/TLR4/NF- κ B inflammatory signaling pathway and UA transporters. The study may provide pharmacological basis to develop and research the new TCM drugs for HUA.

Materials and methods

Chemicals and reagents

LE consists of 25% lard oil (Zhejiang Zhanyi Food Co., Ltd. Zhejiang, China), 10% cholesterol (Shanghai Yuanye Biotechnology Co., Ltd. Shanghai, China), 2% sodium deoxycholate (Shanghai Boao Biotechnology Co., Ltd. Shanghai, China), 0.75% propylthiouracil (Shanghai Chaohui Pharmaceutical Co., Ltd. Shanghai, China), 25% Tween-80 (Shenggong Biological Co., Ltd. Shanghai, China), and 20% propylene glycol (Shandong Yousuo Chemical Technology Co., Ltd. Shandong, China). The UA biochemical reagents kits were purchased from Ningbo Meikang Biotechnology Co., Ltd (Zhejiang, China). The acteoside and astilbin reference substance were provided by Shanghai yuanye Bio-Technology Co., Ltd (Shanghai, China).

Rabbit polyclonal antibodies, including, ABCG2 (4477), GLUT9 (00075489), Toll-like receptor 4 (TLR4) (29g3190), nuclear factor kappa-B (NF- κ B) (00057776), and rat polyclonal antibody beta actin (β -actin) (ZP3822BP22) were purchased from Protein Technology Inc (MA, USA). The enzyme linked immunosorbent assay (ELISA)

kit with lipopolysaccharide (LPS) was purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd (Shanghai, China). Hematoxylin-eosin (H&E) dye solution, 3,3'-Diaminobenzidine (DAB) chromogenic agent, HRP conjugated goat anti-rabbit IgG, Phenylmethanesulfonyl fluoride (PMSF), BCA protein assay kit and 10% SDS-PAGE Resolving Gel were purchased from Beyotime Biotechnology Co., Ltd (Shanghai, China). Radio Immunoprecipitation assay (RIPA) was purchased from Solarbio, Biotechnology Co., Ltd (Beijing, China).

Plant material and preparation of DOS

The Chinese herbs for DOS are weighed in a certain proportion and soaked in distilled water for 10 hours, and then heated reflux twice with distilled water (material-liquid ratio was 1:10, *w/v*) under reflux for 2 hours each time. The high concentration extract of DOS (crude drug concentration was 1.38 g/mL) was concentrated at 65 °C by rotary evaporator, and then the medium and low concentration extract of DOS were obtained from diluting high concentrations extract of DOS with distilled water respectively. (*Dendrobium officinale* was offered by Zhejiang Senyu Co., Ltd).

High performance liquid chromatography analysis of DOS

The Agilent 1260 Series High Performance Liquid Chromatograph equipped with a diode array detector (HPLC-DAD) (Agilent Technologies, Santa Clara, California, USA) was used to identify and determine the main composition of DOS. The chromatography was performed on an Agilent Ultimate LP-C18 (4.6 mm × 250 mm, 5 μm). The mobile phase was methanol (A) and composed of (B) 0.2% formic acid in water (*v/v*), and (B) acetonitrile with the following gradient elution: 0 ~ 5min, 25% A, 75% B; 5 ~ 10min, 25~30% A, 75 ~ 70% B; 10 ~ 25min, 30~40% A, 70 ~ 60% B; 25 ~ 45min, 40 ~55% A, 60 ~ 45% B; 45 ~ 60min, 55~70% A, 45 ~ 30% B; 60 ~ 75min, 70~25% A, 30 ~ 75% B. The flow rate was set at 1.0 mL/min. The temperatures of column and autosampler were maintained at 30 °C and 10 °C, respectively. The injection volume was 10 μL and the detection wavelength was 280 nm.

Animal model of hyperuricemia and drug administration

50 specific pathogens free (SPF) SD rats, with a body mass of 200±20 g, were purchased from JOINN New Drug Research Center Co., Ltd (Jiangsu, China), and housed in a temperature and humidity controlled environment with a 12 h light-dark cycle. All studies were carried out in accordance with the guidelines for the Use and Care of Laboratory Animals published by the Zhejiang province (2009).

After a week of acclimation to the laboratory, 50 male SD rats were randomly and

equally divided into five groups: normal control (NC) group, model control (MC) group, high dose of DOS (DOS-H) group, medium dose of DOS (DOS-M) group, and low dose of DOS (DOS-L) group. The NC group was given an equal volume of 0.5% sodium carboxymethylcellulose solution, and the others groups were orally administered with LE in a volume of 10 mL/kg to establish HUA model of rat each morning, and then three doses of DOS was given with a volume of 10 mL/kg in the afternoon every day during the whole experiment lasting 6 weeks.

Blood samples collection and determination of SUA and LPS

Rats were fasted for 12 hours, then blood samples were collected from the ophthalmic venous plexus at 2nd and 6th weeks, water-bathed for 2 hours at 37 °C, and centrifuged at 3500 r/min for 10 min to obtain the serum to be stored at -20 °C. The SUA level were measured by automatic biochemical analyzer (Hitachi 7020, Japan).

At the end of experiment, all rats were anesthetized (Intraperitoneal injection pentobarbital sodium, a volume of 0.09 g/kg), collected the hepatic portal vein blood, and then sacrificed the rats. Finally, took the intestinal tissues from rats. Blood samples were centrifuged at 3500 r/min for 10 min to obtain the serum to be stored at -20 °C, which were prepared to determine the concentration of LPS in hepatic portal vein blood by ELISA analysis.

Feces sample collection and determination of FUA

Before the end of the experiment, the feces of all rats were collected. Approximate 0.1 g of feces were weighed, mixed with 9 times the volume of PBS, centrifuged at 3500 r/min for 15 min, and the supernatant was collected. The UA level in feces were measured by automatic biochemical analyzer.

Histopathological observation of intestine

The intestine tissues were removed and immediately fixed in 10 % formalin, then, the intestine tissues were embedded in paraffin (MEIKO EC360 Tissue Embedder, Germany). After that, they were prepared to a 4 µm thick paraffin section on a rotary microtome (LEICARM2245 slicing machine, Germany) and stained by H&E method. The histopathological changes of intestine were observed by biological microscope (OLYMPUS BX43, Germany).

Immunohistochemistry (IHC) staining observation

Paraffin embedded intestinal tissues were cut into 4 µm thick sections mounted on glass slides, exposed to 0.5% H₂O₂ for 10 min. The intestinal paraffin sections were then incubated sequentially with rat or rabbit antibody against ABCG2 (dilution 1:200),

GLUT9 (dilution 1:200), TLR4 (dilution 1:200) and NF- κ B (dilution 1:200), horseradish peroxidase-conjugated anti-rat IgG (1:200). The signals were visualized by DAB staining and the nuclei were counterstained with hematoxylin. The results of protein expressions level were evaluated by semi-quantitative analysis as integrated option density (IOD) in positive area of the microphotograph with the Image-Pro Plus software.

Western blot analysis

The intestinal tissues were weighed, put into pre-cooled RIPA buffer containing PMSF and cracked at 4 °C for 1 hour. The intestinal homogenate was centrifuged at a speed of 12000 r/min for 10 minutes, then the supernatant was collected. The protein concentration of the intestinal tissues were detected by BCA assay, then, the protein sample was mixed with the sample buffer in a ratio of 4:1 and boiled for 15 minutes at 100 °C. The protein sample of intestinal tissues were separated from 10% SDS-PAGE and transferred onto PVDF membrane (Bio-Rad, USA), which was then blocked in 5% non-fat milk at room temperature for 2 h. The membrane was incubated with rabbit antibody against ABCG2 (dilution 1:2000), GLUT9 (dilution 1:400) and β -actin (dilution 1:5000) at 4°C overnight, followed with 3x10 min TBS-T washes. After that, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit IgG at 25 °C for 2 hours, and then washed 3x10 min in TBST solution. Finally, the protein bands were displayed by ECL kit, and the optical density of the protein of interest relative to that of β -actin was analyzed by Image J software.

Statistical analysis

All experiment data were expressed as mean \pm standard deviation (SD). SPSS 17 statistical software was used to analyze data. Statistical differences between groups were determined using Student's t-test. $P < 0.05$ was considered as a statistically significant difference, Graphs were performed using performed by Graph Pad Prism 7.0.

Results

HPLC identify and determine the main composition of DOS.

As shown in Figures 1A-C, the two compounds acteoside (peak 1, $R_t=27.472$ min) and astilbin (peak 2, $R_t=28.228$ min) in DOS were identified by HPLC method. The contents of acteoside and astilbin were 1.39 mg/g and 0.72 mg/g, respectively, determined by the each linear-regression equation of standards. These data were

showed in Table 1.

Effects of DOS on SUA level and FUA level in HUA rat.

As shown in Figures 2A-B. After administering LE for 6 weeks, the SUA level in the MC group was significantly increased ($P<0.01$), which indicated that the HUA model of rats were successfully established. The three doses of DOS could remarkably reduce the SUA level at the 2nd week compared with the MC group ($P<0.05$, $P<0.01$), and the degree of decline were 19.30%, 22.38% and 34.12%, respectively. Moreover, after oral administration of DOS for 6 weeks, the degree of decrease in the SUA level were 23.85%, 19.37% and 30.20%, respectively. These results demonstrated that the DOS could significantly and stably decrease the SUA level in rat model of HUA induced by LE.

We also measured the UA level in the feces of HUA rat to observe the excretion of UA level via intestine. The FUA level was significantly decreased by oral administration of LE for 6 weeks ($P<0.01$). As a consequence, the three doses of DOS could remarkably increase the FUA level in the HUA model of rat ($P<0.05$, $P<0.01$). These results suggested that DOS could decrease SUA level in HUA rat through promoting the intestinal excretion of UA level. As shown in Figure 2C.

Effects of DOS on intestinal UA transporter proteins ABCG2/GLUT9 in HUA rat.

As shown in Figures 3A-G. Intestinal excretion of UA is closely associated with the protein levels of UA transporter in the intestine. The protein levels of intestinal ABCG2 and GLUT9 were determined by IHC staining and Western blot assay. Compared with the NC group, the intestinal ABCG2 protein level was markedly reduced ($P<0.01$), but the GLUT9 at protein level in the intestine was remarkably increased in this animal model ($P<0.01$). After oral administration of DOS for 6 weeks, the high and medium doses of DOS could stably decrease protein level of GLUT9, and the three doses of DOS could significantly enhance ABCG2 at protein level in the intestine. These results demonstrated that DOS could inhibit the intestinal reabsorption of UA and promote intestinal excretion of UA through up-regulating ABCG2 protein level and down-regulating GLUT9 protein level in the intestine, and then decrease SUA level in rat model of HUA.

Effects of DOS on intestinal LPS/TLR4/NF- κ B pathway and the histopathological changes of intestine in HUA rat.

Next, we further investigated the protective effects of DOS on intestinal inflammation responses in rat model of HUA. HE staining showed that the intestinal

epithelial cells in the NC group had a complete structure and were neatly-arranged, however, the number of intestinal glandular goblet cells of rat with hyperuricemia was significantly decreased, and the small intestinal epithelial cells were partially shed. After administration of DOS for 6 weeks, both high and medium doses of DOS increased the number of intestinal gland goblet cells, and the small intestinal epithelial cells have complete structure, which are arranged neatly in HUA rat. As shown in Figure 4A.

LPS is a lipopolysaccharide located in the cell wall of Gram-negative bacteria^[32], which can activate the TLR4/NF- κ B inflammatory response^[33], secrete inflammatory cytokines, and cause damage to the intestine. As shown in Figure 4B, the concentration of LPS was significantly increased in hepatic portal vein blood by oral administering with LE ($P<0.05$). However, the three doses of DOS significantly reduce the content of LPS in hepatic portal vein blood after administration of DOS for 6 weeks ($P<0.05$). It is suggested that DOS improved the injury of inflammatory responses to the intestine in rat model of HUA.

The intestine^[34, 35] is responsible for 30% of UA excretion in the body, and its histopathological changes may cause HUA^[36, 37]. In this experiment, the protein levels of TLR4 and NF- κ B in the intestine of the HUA rat were remarkably increased ($P<0.05$). The medium and high doses of DOS significantly decrease the intestinal TLR4 and NF- κ B protein levels in the rat model of HUA ($P<0.05$). As shown in Figures 4C-F. These results indicated that DOS obviously ameliorated LE-induced intestinal inflammatory injury in rat through down-regulating protein levels of TLR4 and NF- κ B.

Discussion

Rat was administered with LE^[30], which can increase the production of low-density lipoprotein cholesterol^[38] (LDL-c) and promote the de novo synthesis of purine nucleotides from ribose 5-phosphate to 5-phospho-ribosyl pyrophosphate. In addition, hypoxanthine and xanthine are in turn the products of purine metabolism starting from ribose-5-phosphate^[39], it will eventually lead to an increase in the production of UA.

Another study found that high cholesterol level can also cause oxidative stress^[40, 41], which can promote the feedforward cycle of uric acid production and eventually lead to an increase in serum uric acid level^[42, 43]. In addition, lipid metabolism disorder may involve afferent and efferent arterioles^[44, 45], resulting in vascular stenosis or occlusion, and then inhibiting renal excretion of UA and increasing the level of SUA.

In early laboratory research, Min-xia Pang^[30] and Ying-ying Ma^[31] found that HUA model of rat can be successfully established through administering LE for 6 weeks, and the HUA model is more stable and sustainable than others.

In the present study, HUA was successfully induced by administering LE for 6 weeks in rat, which indicated that LE could be used to study the causes and pathogenesis of metabolic abnormalities related to HUA, and select the new anti-hyperuricemic Chinese medicine in pharmacological research. We found that the FUA level in the MC group was remarkably lower than that in the NC group, indicating that there was an obstacle in the intestinal excretion of UA in the rat model of HUA. After administration of DOS for 6 weeks, DOS could markedly decrease the SUA level that the largest degree of decline up to 34.12%, and increase the FUA level in the HUA model of rat induced by LE. These results showed that DOS could decrease SUA level through promoting the intestinal excretion of UA level in rat model of HUA.

Production and excretion of UA are balanced in the normal human body, the average UA pool is approximate 1200 mg, producing 750 mg of UA per day, and excreting 500 to 1000 mg^[30] every day. The intestine is the largest organ for excretion of UA except for the kidney, about 30% of the daily UA is excreted from the intestine into feces. ABCG2 is a high-capacity uric acid exporter, which is more highly expressed in small intestinal epithelial cells than in proximal renal tubular cells. Multiple studies^[46-48] have shown that increased SUA level is associated with inhibiting the activity of ABCG2 protein. Yano et al^[49] showed that 5/6 nephrectomy rat exhibited lower excretion of UA in the urine and overexpression of ABCG2 in the ileum, while SUA did not significantly increase, which suggests that an excretory pathway other than the kidney, probably the intestine, may operate in a complementary role. In summary, ABCG2 is essential for intestinal excretion of UA.

GLUT9 is a voltage sensitive urate transporter, mainly expressed in the kidneys, liver and intestine, which can reabsorb the UA into the blood^[50], and then increase the SUA level in the body. In this study, after oral administration of DOS for 6 weeks, DOS significantly elevated FUA level to reduce SUA level in rat model of HUA induced by LE possibly through up-regulating intestinal ABCG2 protein level and down-regulating intestinal GLUT9 protein level. These results demonstrated that DOS could inhibit the intestinal reabsorption of UA and promote intestinal excretion of UA and then decrease SUA levels via intestine, further confirmed the anti-hyperuricemia effect of DOS.

Improving intestinal lesions may increase intestinal excretion of UA. There are

several studies^[51-53] have shown that high-fat diets are associated with a reduction in intestinal bacterial diversity and increasing in permeability of cell membrane. This facilitates the passage of bacteria and LPS through the intestinal wall. LPS goes to chylomicrons and through the portal vein enters the bloodstream, and then LPS-mediated TLR4/NF- κ B signaling pathway^[54-56] and an increased production of proinflammatory cytokines, causing a systemic inflammatory response which can damage the intestine and aggravate intestine dysfunction. In this study, DOS could significantly decrease the concentration of LPS in hepatic portal vein blood after administration for 6 weeks, and the expression levels of TLR4 and NF- κ B protein in the intestine of the HUA rat remarkably decreased after administration of DOS for 6 weeks, and H&E staining showed DOS markedly improve the histopathological changes of intestinal tract in HUA rat. These results further confirmed the intestinal protective effects of DOS through improving the injury of inflammatory response to intestine by inhibiting LPS/TLR4/NF- κ B signaling pathway in the animal model with HUA.

In our study, the acteoside and astilbin were the main compositions in DOS. There is a study^[57] demonstrated that the SUA level was significantly decreased by increasing the urine UA (UUA) level and fractional excretion of UA (FEUA) by oral administration of astilbin, and another study showed that acteoside^[58] could reduce SUA level and inhibit XOD activity. It indicated acteoside and astilbin are potential and effective component of DOS with UA lowering effect.

Conclusion

In summary, the lowering effect of DOS on SUA level and promoting effect on intestinal excretion of UA were confirmed in the HUA rat induced by LE. The possible molecular mechanism may relate to inhibit LPS/TLR4/NF- κ B signaling pathway in the intestine by oral administration of DOS, reduce inflammatory responses to improve the intestinal histopathological changes, and then promote intestinal excretion of UA and inhibit intestinal reabsorption of UA to decrease the UA level. This study can provide a pharmacological basis for the research and development of new TCM to treat HUA.

Abbreviations

DOS: *Dendrobium officinale* Six nostrum; UA: uric acid; HUA: hyperuricemia; LE: lipid emulsion; SUA: serum uric acid; FUA: fecal uric acid; LPS: Lipopolysaccharide; ABCG2: ATP-binding cassette superfamily G member 2; GLUT9: glucose transporter 9; TLR4: Toll-like receptor 4; NF- κ B: nuclear factor kappa-B; IHC: immunohistochemistry; H&E: Hematoxylin and eosin staining; HPLC: High Performance Liquid Chromatography; XOD: xanthine oxidase; UUA: urine uric acid; FEUA: fractional excretion of UA.

Acknowledgements

Not applicable.

Authors' contributions

Shan-Shan Lei and Bo Li designed the study. Xue Chen, Hong-Zhang Ge and Zhe-Tian Jiang participated in the *Dendrobium officinale* Six nostrum administration and pharmacology research. Jie Su and Qiao-Xian Yu drafted the manuscript. Su-Hong Chen and Gui-Yuan Lv supervised the study. All authors approved the final manuscript.

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Availability of data and materials

The research data generated from this study is included within the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Table 1. Standard linear-regression equation and content of two main compounds in DOS

Compound	Linear-regression equation	R²	Linearity rage (µg/mL)	Content (mg/g)
Acteoside	Y=10.404x-52.53	0.9985	5-400	1.39
Astilbin	Y=21.436x	0.9999	5-400	0.72

Figure Legend

Fig.1 Standard liquid chromatogram of acteoside (A) and astilbin (B), main compounds liquid chromatogram in DOS (C).

Fig.2 DOS could improve SUA level of the HUA rat at the 2nd and 6th weeks. (A) SUA level after administration of DOS for 2 weeks, (B) SUA level after administration of DOS for 6 weeks. (C) FUA level after administration of DOS for 6 weeks.

Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean \pm SD (n=9-10). [#]*P*<0.05; ^{##}*P*<0.01, compared with NC; **P*<0.05; ***P*<0.01, compared with MC group.

Fig.3 DOS increased the expression level of ABCG2 protein and reduced the expression level of GLUT9 protein in the intestine of HUA rat after administration for 6 weeks. (A and B) expression of ABCG2 in intestine by IHC (\times 400) and semi-quantitatively analysed as integrated option density (IOD) in positive area of the microphotograph. (C and D) expression of GLUT9 in intestine by IHC (\times 400) and semi-quantitatively analysed as IOD in positive area of the microphotograph. (E-G) expression of ABCG2 protein and GLUT9 protein in intestine by Western blot assay and were normalized to β -actin.

Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean \pm SD (n=9-10). [#]*P*<0.05; ^{##}*P*<0.01, compared with NC; **P*<0.05; ***P*< 0.01, compared with MC group.

Fig.4 DOS improved LPS/TLR4/NF- κ B pathway and the histopathological changes of intestine in HUA rat after administration for 6 weeks. (A) Intestinal histopathological changes were directly reflected by H&E staining (\times 200), a: Intestinal epithelial cells; b: Intestinal gland goblet cells. (B) the content of LPS in hepatic portal vein blood by ELISA. (C and D) expression of TLR4 in intestine by IHC (\times 400) and semi-quantitatively analysed as IOD in positive area of the microphotograph. (E and F) expression of NF- κ B in intestine by IHC (\times 400) and semi-quantitatively analysed as IOD in positive area of the microphotograph.

Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean \pm SD of (n=9-10). [#]*P*<0.05; ^{##}*P*<0.01, compared with NC; **P*<0.05; ***P*<0.01, compared with MC group.

Fig.1

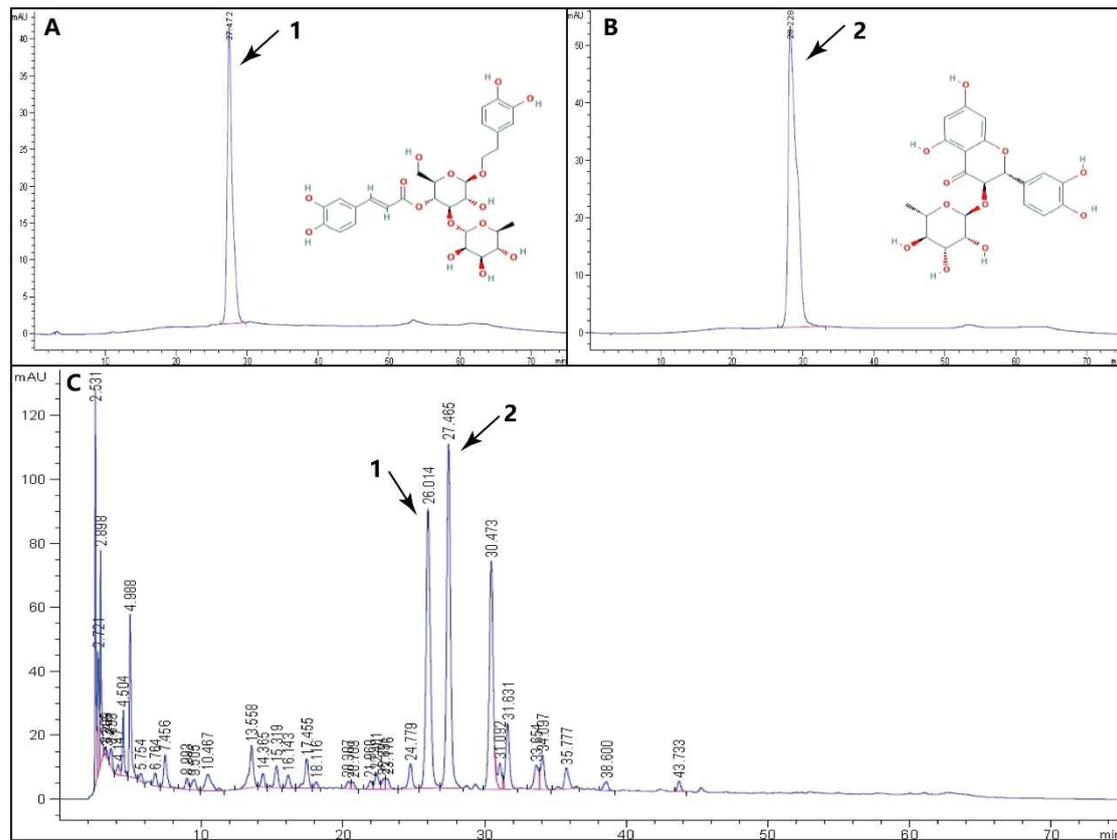


Fig.2

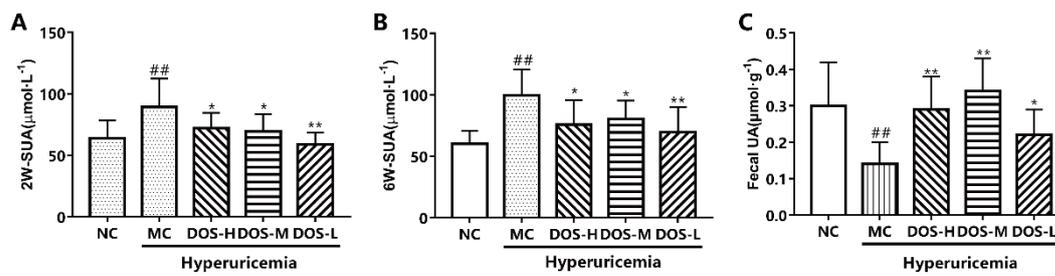


Fig.3

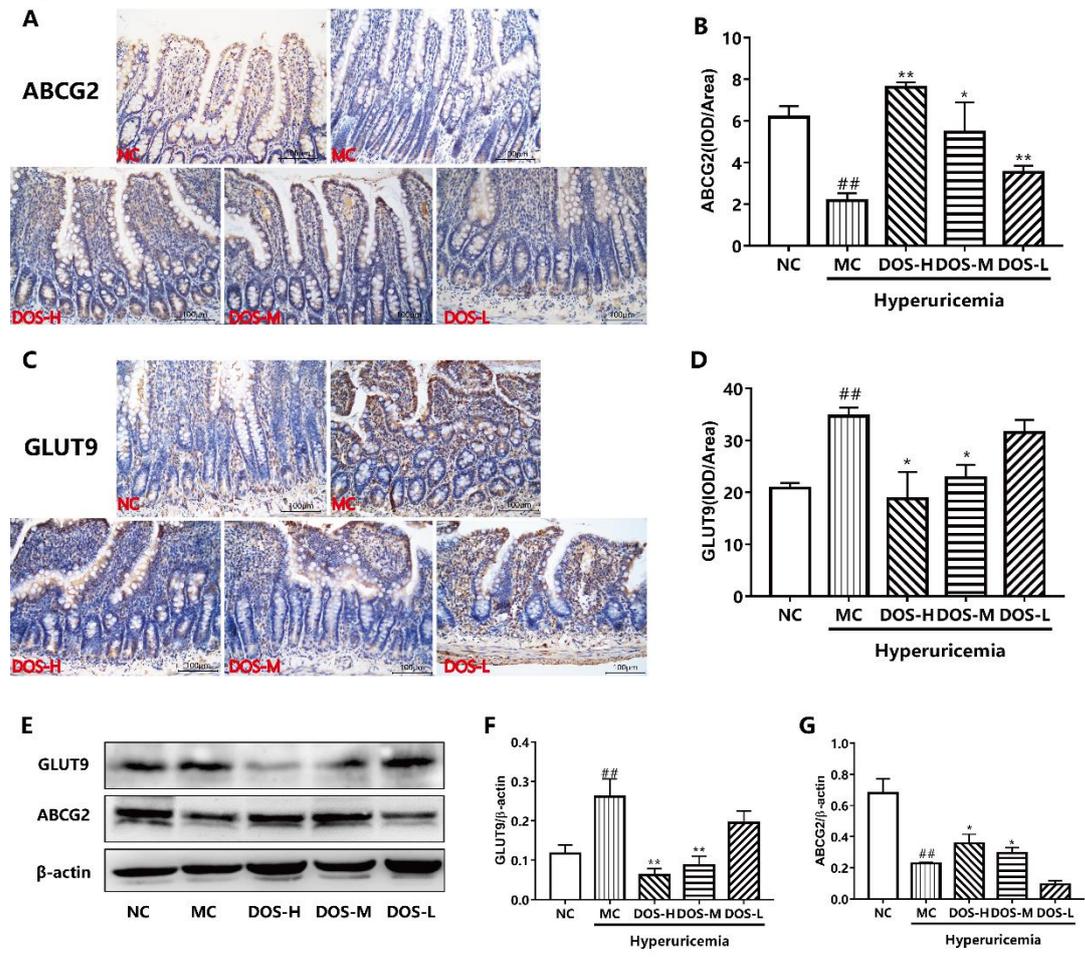
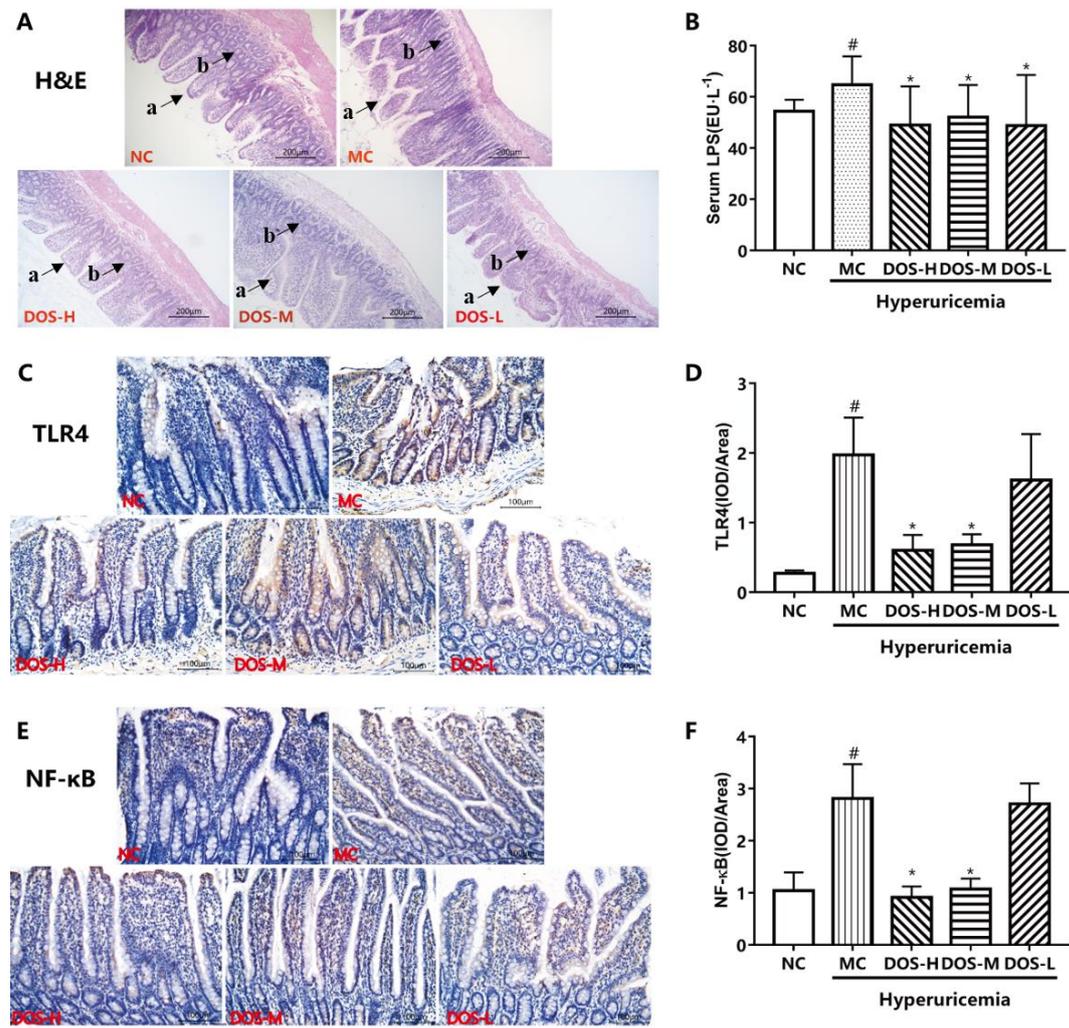


Fig.4



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Figures

Fig.1

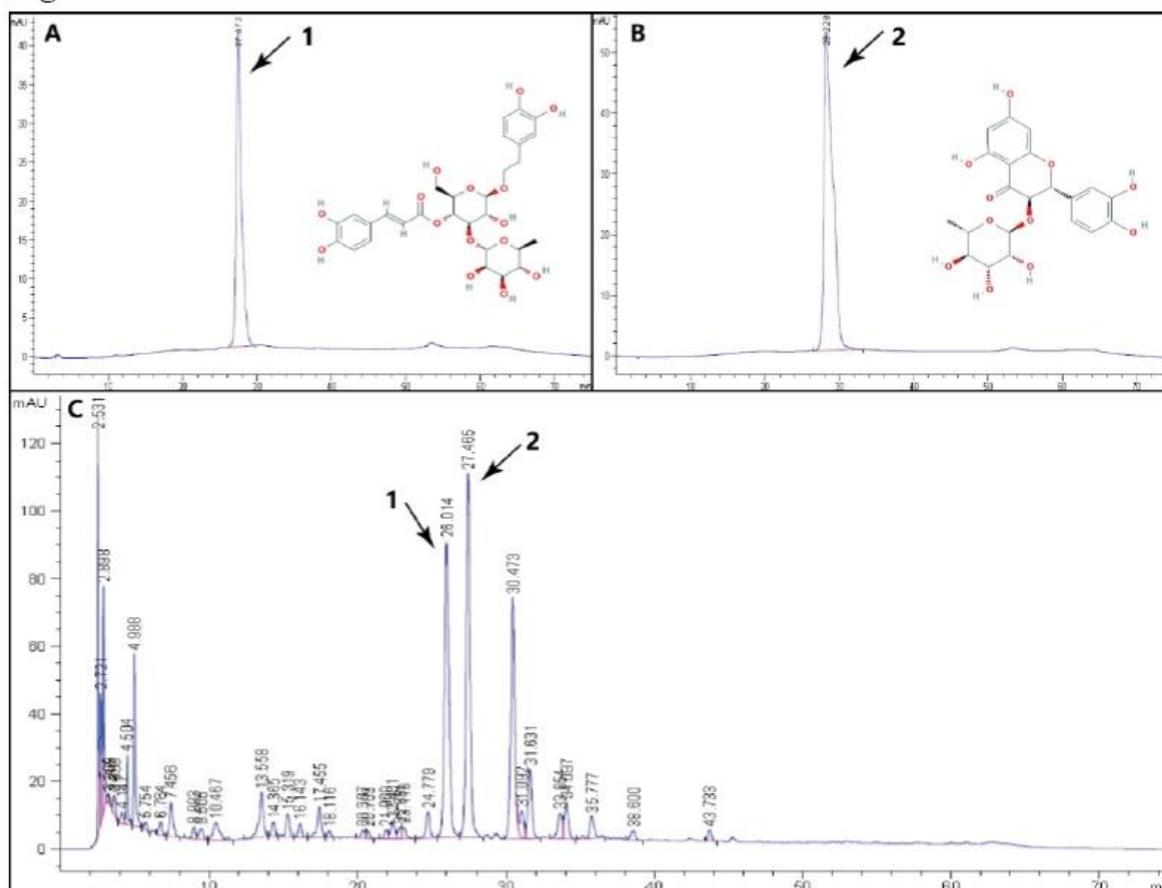


Figure 1

Standard liquid chromatogram of acteoside (A) and astilbin (B), main compounds liquid chromatogram in DOS (C).

Fig.2

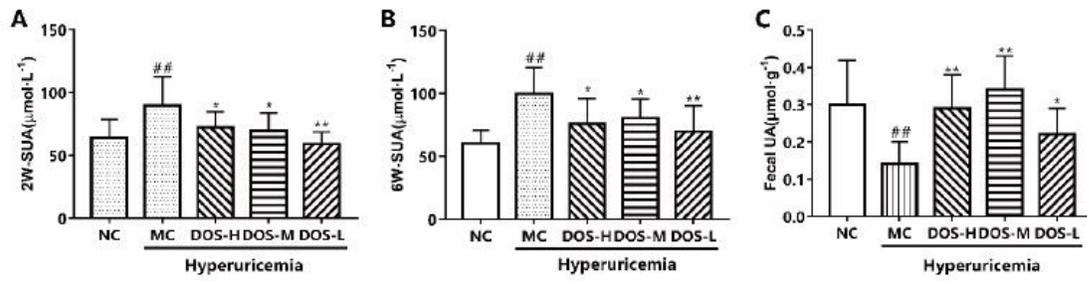
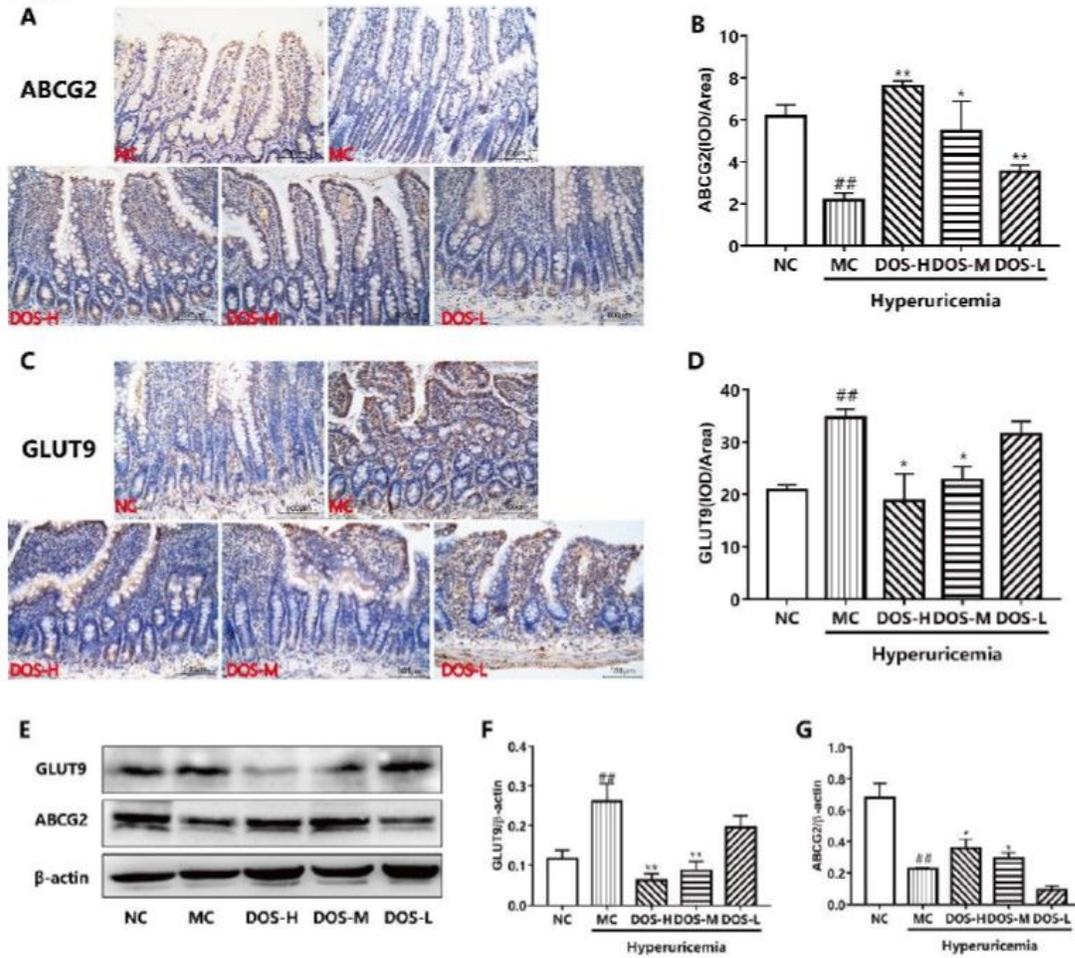


Figure 2

DOS could improve SUA level of the HUA rat at the 2nd and 6th weeks. (A) SUA level after administration of DOS for 2 weeks, (B) SUA level after administration of DOS for 6 weeks. (C) FUA level after administration of DOS for 6 weeks. Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean \pm SD (n=9-10). #P<0.05; ##P<0.01, compared with NC; *P<0.05; **P<0.01, compared with MC group.

Fig.3**Figure 3**

DOS increased the expression level of ABCG2 protein and reduced the expression level of GLUT9 protein in the intestine of HUA rat after administration for 6 weeks. (A and B) expression of ABCG2 in intestine by IHC ($\times 400$) and semi-quantitatively analysed as integrated option density (IOD) in positive area of the microphotograph. (C and D) expression of GLUT9 in intestine by IHC ($\times 400$) and semi-quantitatively analysed as IOD in positive area of the microphotograph. (E-G) expression of ABCG2 protein and GLUT9 protein in intestine by Western blot assay and were normalized to β -actin. Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean \pm SD (n=9-10). ^{##}P<0.05; ^{##}P<0.01, compared with NC; ^{*}P<0.05; ^{**}P< 0.01, compared with MC group.

Fig.4

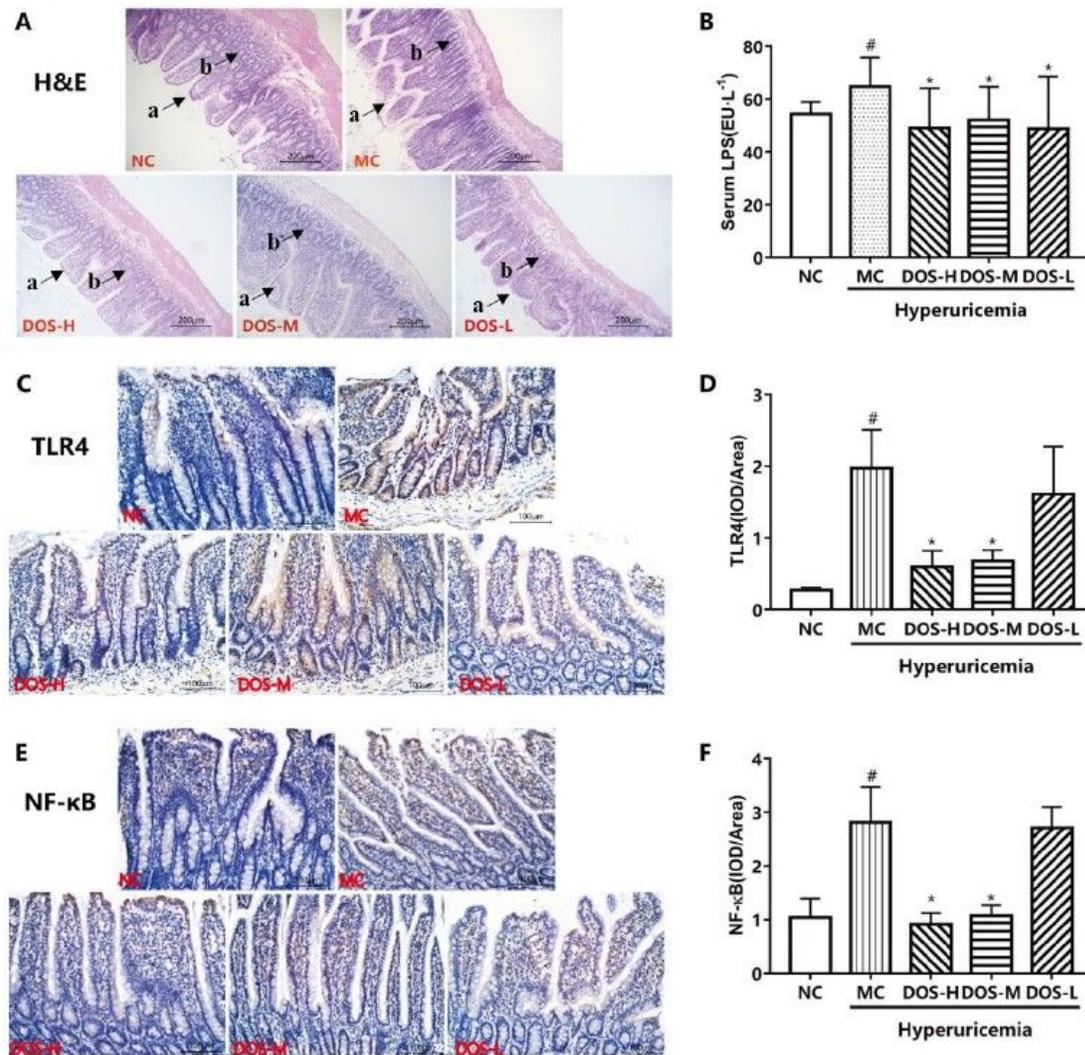


Figure 4

DOS improved LPS/TLR4/NF-κB pathway and the histopathological changes of intestine in HUA rat after administration for 6 weeks. (A) Intestinal histopathological changes were directly reflected by H&E staining (×200), a: Intestinal epithelial cells; b: Intestinal gland goblet cells. (B) the content of LPS in hepatic portal vein blood by ELISA. (C and D) expression of TLR4 in intestine by IHC (×400) and semi-quantitatively analysed as IOD in positive area of the microphotograph. (E and F) expression of NF-κB in intestine by IHC (×400) and semi-quantitatively analysed as IOD in positive area of the microphotograph. Notes: NC-normal control group; MC-model control group; DOS-L-low dose of DOS; DOS-M-medium dose of DOS; DOS-H-high dose of DOS. The data were expressed as mean ± SD of (n=9-10). #P<0.05; ##P<0.01, compared with NC; *P<0.05; **P<0.01, compared with MC group.