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Juglone prevents metabolic endotoxemia-induced hepatitis and neuroinflammation via suppressing TLR4/NF-κB signaling pathway in high-fat diet rats

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ABSTRACT

Juglone as a natural production mainly extracted from green walnut husks of Juglans mandshurica has been defined as the functional composition among a series of compounds. It showed powerful protective effect in various diseases by inhibiting inflammation and tumor cells growth. However, studies on its anti-inflammatory effect based on high-fat diet-induced hepatitis and neuroinflammation are still not available. In this regard, we first investigated whether juglone suppresses high-fat diet-stimulated liver injury, hypothalamus inflammation and underlying mechanisms by which they may recover them. SD rats were orally treated with or without high-fat diet, 0.25 mg/kg or 1 mg/kg juglone for 70 days. Subsequently, blood, hypothalamus and liver tissue were collected for different analysis. Also, the primary astrocytes were isolated and used to analyze the inhibitory effect of juglone in vitro. Analysis of inflammatory cytokines declared that the inhibition of TNF-α, IL-1β and IL-6 could be carried by juglone in response to high-fat diet rats. Meanwhile, TLR4 expression and NF-kappa activity also have been confirmed to be the key link in the development of hepatitis and nerve inflammation. The activation was significantly suppressed in treatment group as compared with model. These results indicated that juglone prevents high-fat diet-induced liver injury and nerve inflammation in mice through inhibition of inflammatory cytokine secretion, NF-kappa B activation and endotoxin production.

1. Introduction

Obesity and type II diabetes as the major health problem for all over the world are becoming a modern disease, linked to cancer, high blood pressure, heart disease, hepatitis and other health problems [1,2]. Globalization of unhealthy lifestyles like unhealthy diets may show up in individuals as raised blood pressure, increased blood glucose, elevated blood lipids, overweight and obesity [3,4]. Both cancer and heart disease are conditions with multiple causes such as genetics, smoking, obesity and diet [5]. According to the data of WHO showed that being overweight is normal but the 2.5 million cases of obesity-related diabetes are posing an enormous threat to health. Among the factors, the global confluence of changing diets (especially for high-fat diet), increased food marketing, access to technology and lifestyle changes has led to an upsurge in global obesity, especially in developing countries [6,7]. However, the key link of high-fat intake induced obesity is associated with the action of gut microbiota changes [8]. Relation of intergrowth and prey of good bacteria and maleficent bacteria in animals' body have effect for body health. A lot of researches have illustrated that high-fat diet has ability to enhance maleficent bacteria production [9]. Maleficent bacteria can cause mass toxin (LPS), when maleficent bacteria of intestinal ecosystem take absoluteness vantage, can cause grave disease for body organ [9,10]. The abundant endotoxin released from bacteria will penetrate intestinal epithelium into blood to cause intestinal endotoxemia. No doubt that intestinal endotoxemia is deemed to be a major reason for nonalcoholic fatty liver disease (NAFLD) and neuroinflammation of hypothalamus that is response to feeding behavior [11,12] (Fig. 1A). Also, the related report indicated that hepatitis is capable of stimulating the development and improvement of nerve injury [13,14].

Of note, the persistent replication of inflammation is closely related to the progression of infection and the occurrence of Toll like receptor 4 (TLR4) mediated inflammatory response [15,16]. Lots of researches indicated the important role of TLR4 in

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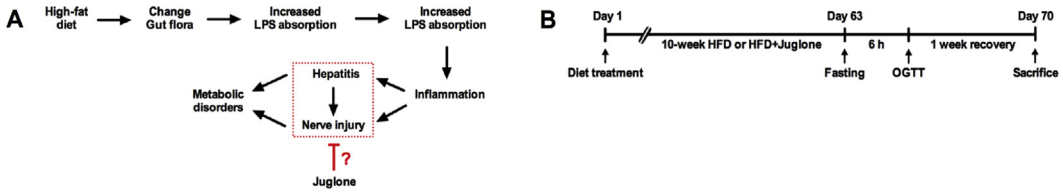

Fig. 1. The possible mechanism of juglone in high-fat diets-induced metabolic disorders and the experimental design. (A) High-fat diets improve alteration of intestinal flora and further enhance increase of endotoxin level to cause metabolic disorders. The dotted box shows the possible mechanism of inhibitory effect of juglone on hepatitis and nerve injury. (B) The experimental design we used in present study.

lipopolysaccharide (LPS) inflammation [16]. The receptor for LPS, a significant mediator of the inflammatory response to infection or poison, plays a key role in the development of a number of human diseases, including hepatitis, cancer and nerve injury [17]. LPS could stimulate interaction between TLR4 and MyD88/TAK1, and further activate NF-κB signaling pathways to activate inflammatory response and inflammatory cytokines release [18]. Hence, how to effectively suppress TLR4 mediated inflammatory signaling pathway might be the key target for drug screening to recover LPS induced inflammation. Although with the development of knowledge in therapy and diagnosis, the efficient treatment method and drugs remains not fully understood and explained.

Lately, juglone (Jug), a natural plant product extracted from green walnut husks of Juglans mandshurica, a deciduous tree widely cultivated in India, China and Indochina, has displayed potential anti-mycobacterials activity and antitumor activity [19]. Juglone as an important source of naphthoquinone drugs, the fruits have been widely used in folkloric statement of China for the precaution of various human diseases, including cancer, stomach pain and angiocardiopathy [19,20]. However, there are no related reports about the anti-inflammatory effect of juglone and its mechanism. Hence, we used high-fat diet rats to investigate the anti-inflammatory effect of juglone on hepatitis and nerve injury.

2. Materials and methods

2.1. Animals and drugs administration

The 6–8 weeks male SD rats weighing 150 g–180 g were obtained from the Shanghai experimental animal center (Shanghai, China) and kept in a temperature and humidity-controlled environment (25 ± 2 °C, 50 ± 10% humidity) with a standard 12 h light and 12 h dark cycle with food and water in their cages. All processes were in accordance with the Institutional Animal Care and Use Committee of Guilin Medical University. Juglone (CAS: 481-39-0, purity ≥ 98%) were purchased from Chengdu Preferred Biological Technology Co., LTD (Chengdu, China) and prepared in PBS or DMSO. Research Diets D12492i 60 kcal% Fat was obtained from Open Source Diets, Inc. (New Jersey, USA). All rats were randomly divided into 4 groups: (1) Control; (2) High-fat diet group (HF); (3) Low dose of 0.25 mg/kg Juglone + HF (L-Jug); (4) High dose of 1 mg/kg Juglone + HF (H-Jug). Rats were orally administrated with different concentration of juglone. The detail procedures of experiments have been illustrated in Fig. 1B.

2.2. ELISA measurement and biochemical analysis

After the last administration and extracting the eyeball blood, inflammatory cytokines were investigated using ELISA kits in accordance with the manufacturer's instructions (R&D system, USA). Besides, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also analyzed using a Diagnostics ALT and AST test kit from Sigma–Aldrich (Cat. No MAK052, MAK055, St. Louis, Missouri, USA) and automatic biochemical analyzer (Hitachi Auto Analyzer 7170, Japan). Also, the oral glucose tolerance test (OGTT) (2 g/kg glucose) was conducted according to test methods of Lorenzo et al. [21]. All of the other indicators listed in Table 1 were measured using Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) kits according to the production specification.

2.3. Primary astrocytes culture and MTT analysis

The preparation of primary astrocytes were isolated and cultured in accordance with Canki et al. [22]. method with some modification. In brief, the 2–3 days neonatal rats were sterilized using 75% alcohol. And then whole hypothalamus was absolutely isolated and maintained in cold Hanks solution. The 0.25% lysine-enzymatic digestion and tissue pieces were designed for culturing astrocytes from newborn rats. Subsequently, all the cells were centrifuged at 1000 rpm for 5 min to remove debris and impurities and then were plated in 6-well plates. The MTT analysis is accordance with Zhu et al. [23]. and studies the effect of time-dependent and dose-dependent on cell viability.

2.4. H&E, red oil and immunohistochemical staining

The collections of live samples were subjected to H&E staining and were examined for liver injury by light microscopy. In brief, tissues were fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4). After dehydration, thin sections were strictly evaluated under light microscopy. The red oil sections were performed by Guilin Zhenda biotechnology, Co., LTD (Guilin, China). Hypothalamus samples also were subjected to immunohistochemical staining according to R&D introduction and performed by Guilin Zhenda biotechnology, Co., LTD (Guilin, China).

2.5. Quantitative real-time PCR (qPCR)

Total RNA isolation system of Omega Bio-Tek, Inc. was used to isolate total RNA of hypothalamus samples. Briefly, 1 μg of total RNA was reverse transcribed using the M-MLV-RT system. The system was carried at 43 °C for 1 h and stopped by inhibition of the enzyme at 70 °C for 10 min. qPCR were administrated using SYBR Green kits (Bio-Rad) in ABI PRISM 7900HT detection systems (Applied Biosystems). Invitrogen Corporation produced all sequences of primers for qPCR.

2.6. Western blot analysis

All the liver, hypothalamus tissues constitution homogenate and three-passage of cells were administrated with or without 5, 10, 15, 20 umol/L juglone were carried on according to a standard procedure as the protocol reported [12]. Same amount of total protein were clapped into10% or 12% SDS-PAGE followed by immunoblotting using the following antibodies (1:1000): rabbit anti- NF-κB,

Table 1
Effects of juglone on the general parameters in high fat-fed rats.

Event	Control	HF	L-Jug	H-Jug
Body weight gain (g)	220.2 ± 10.13	321.8 ± 8.31$^{\#\#}$	274.1 ± 9.11*	250 ± 10.19**
Fat(% of body weigh)	10.9 ± 1.02	22.1 ± 0.91$^{\#\#}$	15.3 ± 0.49*	13.0 ± 1.17**
Serum TC(mg/dL)	42.2 ± 1.05	70.1 ± 3.01$^{\#\#}$	66.3 ± 1.09	47.8 ± 2.04**
Serum TG(mg/dL)	123.3 ± 5.91	241.1 ± 12.04$^{\#\#}$	211.1 ± 10.02	144.3 ± 10.15**
Serum HDL(mg/dL)	13.1 ± 1.71	34.8 ± 1.99$^{\#\#}$	20.0 ± 2.42*	14.9 ± 3.11**
Serum LDL(mg/dL)	43.9 ± 2.15	25.7 ± 1.4$^{\#\#}$	30.7 ± 3.31	40.8 ± 2.21**
Serum glucose (mmol/L)	7.8 ± 1.16	11.4 ± 0.93	8.1 ± 2.01	7.9 ± 1.71
Serum insulin (ug/mL)	1.79 ± 0.61	3.05 ± 0.70$^{\#\#}$	2.28 ± 0.25*	1.81 ± 0.14**
Serum ALT (U/mL)	16.45 ± 2.12	34.71 ± 1.95$^{\#\#}$	25.19 ± 1.61*	19.49 ± 2.06**
Serum AST (U/mL)	67.83 ± 4.38	81.39 ± 4.12$^{\#\#}$	52.67 ± 3.49**	48.62 ± 4.05**
Serum SOD (U/mL)	56.55 ± 2.46	43.38 ± 5.81$^{\#}$	45.26 ± 2.17	55.23 ± 3.51*
Serum MDA (umol/L)	4.19 ± 1.05	5.57 ± 1.22$^{\#}$	3.36 ± 0.16**	3.94 ± 1.28*
Serum endotoxin (LPS) (EU/mL)	4.03 ± 0.15	48.71 ± 3.24$^{\#\#}$	36.22 ± 2.10*	21.92 ± 1.96**

Mean ± SEM. (n = 10). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ *vs* control rats. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* high fat-fed rats.

TAK1, TLR4, IκBα, MyD88, (Cell Signaling Technology, Inc., USA). Western blot bands were observed using GE Healthcare ECL Western Blotting Analysis System and exposed to x-ray film of Kodak.

2.7. Statistical analysis

The differences of index are evaluated by means ± SEM. Treated tissue and the corresponding controls were compared using Graph Pad PRISM (version 6.0; Graph Pad Software, USA) by a one-way ANOVA with Dunn's least significant difference tests or Student's *t* test. Differences between groups were considered significant at $p < 0.05$. The bars indicate the means ± SEM. (n = 10). #p < 0.05, ##p < 0.01, ###p < 0.001 vs. control group. *p < 0.05; **p < 0.01; ***p < 0.001 vs. HF.

3. Results

3.1. Juglone inhibited high-fat diet-induced liver injury in mice

Rats were treated with high-fat diet, followed by biochemical and histological evaluation of the liver injury. As anticipated in Table 1, serum levels of ALT and AST, both of which are well-established markers of liver damage, were remarkably increased in HF group mice. In addition, serum levels of TG, TC, HDL and MDA were significantly enhanced in HF-fed rats, compared with control. In contrast, low or high dose of juglone treatment rats were reduced in these indicators. The SOD and LDL also could be inhibited during the period of HF diet. Importantly, LPS as the significant indicator of serum endotoxin to evaluate the levels of endotoxemia illustrated that HF diet are capable of increasing LPS level, the result was consist with the reported conclusion and proven to improve liver injury and inflammatory responses. Moreover, juglone has ability to decrease bodyweight in the long-term HF-diet rats. And control rats are more resistant to the HF rats, compare to all groups with AUC (Fig. 2A and B). In juglone treated group, the extent of necrosis and adipose hollow space was reduced obviously. Of note, the elevation of liver tissue TG and TC as well as the hepatic necrosis area in model group were significantly higher than those in treatment group (Fig. 2C and D). Histological analysis also demonstrated that representative hepatic lipid accumulation in HF group could be observed (Fig. 2E).

3.2. Effect of juglone on the mRNA and production of pro-inflammatory cytokines in HF diet rats

Pro-inflammatory cytokines play a significant role in HF diet-induced neuroinflammation of hypothalamus. A lot of researches have given the fact that HF diet could cause a number of cytokines release and inflammatory pathway activation. As expected, our results in Fig. 3 have illustrated that the dynamic changes of main pro-inflammatory cytokines in protein level between 1 week and 10 week in serum and tissues. We found that HF diet significantly increase the pro-inflammatory cytokines levels and promote them release. In contrast, juglone treatment could suppress the responses, compared to HF diet group. In addition, we also investigated the protective effect of juglone on mRNA levels. The results showed that both pro-inflammatory cytokines mRNA and major maker of gliocytes-GFAP and CD11b were markedly enhanced and activated with the increase of fed time.

3.3. Juglone prevented NF-κB signaling pathway in HF diet rats

In this study, to confirm whether juglone significantly inhibited astrocytes activation via suppressing NF-κB signal pathway activation in HF diet-induced inflammatory responses, we observed the major members were involved in NF-κB signaling pathway such as TLR4, MyD88, NF-κB and IκBα expression level. Interestingly, GFAP as the major maker of astrocytes were significantly increased in hypothalamus of HF-fed rats, compared with other groups (Fig. 4A). The GFAP positive cells are higher than the groups in juglone treatment. Also, the immunofluorescence results of hypothalamus indicated that p−NF−κB was largely activated in HF diet rats and inhibited by juglone administration (Fig. 4B). Besides, we also investigated the IκBα expression, which is the key link indicator in TLR4/NF-κB inflammatory signaling pathways. Our data showed that HF diet has ability to increase the degradation of IκBα, compared with juglone-administrated groups. In contrast, the juglone may significantly suppress its level of degradation and phosphorylation (Fig. 4C). Moreover, western blot analysis of astrocytes in hypothalamus demonstrated that HF diet administration could activate the expression of TLR4, MyD88 and TAK1, compared with juglone groups. Meanwhile, juglone treatment significantly inhibited the p−NF−κB and p-IκBα level. All the data in this part illustrated that juglone significantly directly or indirectly inhibited HF diet-induced hypothalamus inflammatory responses via suppression of TLR4/NF-κB signaling pathways.

3.4. Effect of juglone on LPS-induced primary astrocytes inflammatory responses

In this part, we used primary astrocytes to study the inhibitory effect of juglone on LPS-induced astrocytes activation and inflammatory responses. First of all, we test the possible chronic toxicity of juglone on astrocytes. MTT method was used to determine the effect of juglone on time-dependent and dose-dependent in

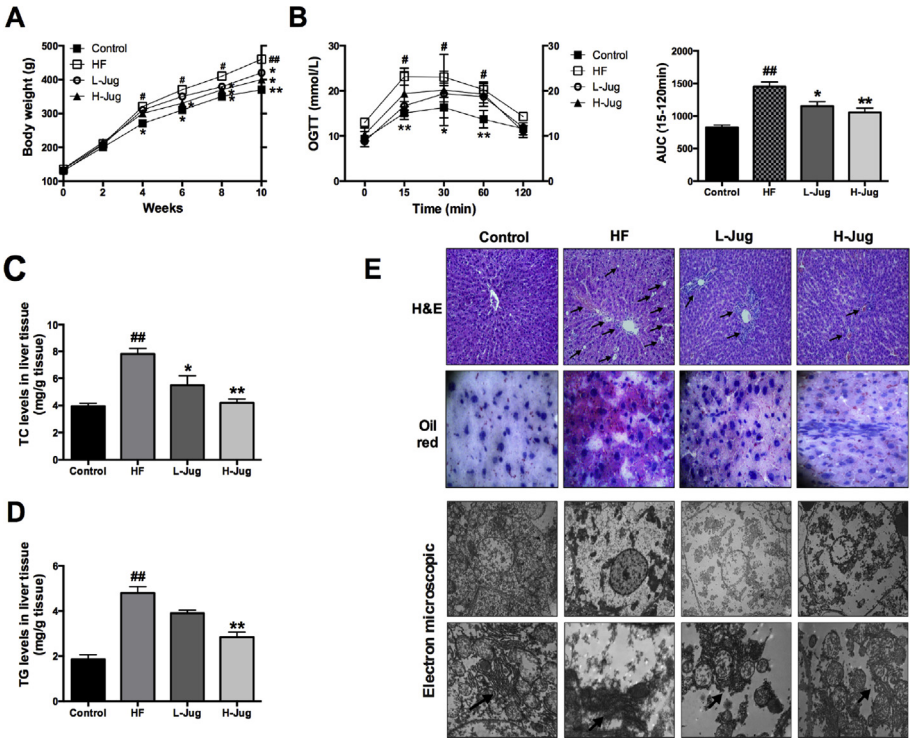

Fig. 2. Inhibitory effect of juglone on hepatitis in high-fat diets rats. (A) Body weight of rats in the period of experiment. (B) Oral glucose tolerance test (OGTT) of different groups from 0 min to 120 min; AUC: area under curve. (C, D) The analysis of TG and TC concentration in liver tissue of HFD rats. (E) Histological analysis of H&E, red oil staining and electron microscopic image for liver tissue.

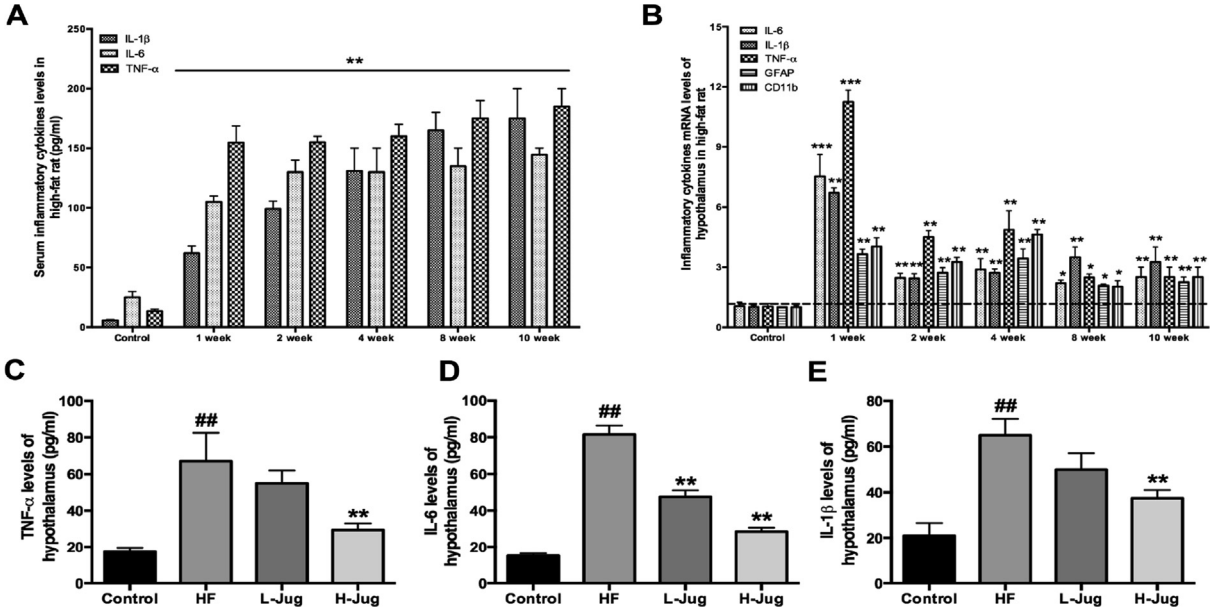

Fig. 3. Juglone suppresses pro-inflammatory cytokines release and mRNA expression in hypothalamus. (A) The dynamic changes of TNF-α, IL-1β and IL-6 production in serum between 1 week and 10 week. (B) The dynamic changes of TNF-α, IL-1β and IL-6 mRNA expression in hypothalamus tissues. Besides, GFAP and CD11b as the glials maker also have been showed to evaluate glials activation. (C–E) TNF-α, IL-1β and IL-6 production of hypothalamus were analysis be ELISA method to compare the inhibitory of juglone on HFD-induced hypothalamus inflammation.

juglone treated astrocytes. As expected in Fig. 4D and E, the data from MTT showed that the highest dose of juglone (30 umol/L) we used in this part has not obvious toxicity could be observed. And in the dose-dependent of 24 h treatment, there is no apparent toxicity could be detected. Also, the results in Fig. 4E indicated that HF diet significantly increase TLR4/NF-κB signaling pathways activation in western blot bands. In contrast, compared with model mice, juglone treatment significantly reduces TLR4/NF-κB related indicators expression and activation.

4. Discussion

Without a doubt, along with the improved living standard, many nations now record more than 20 percent of their population

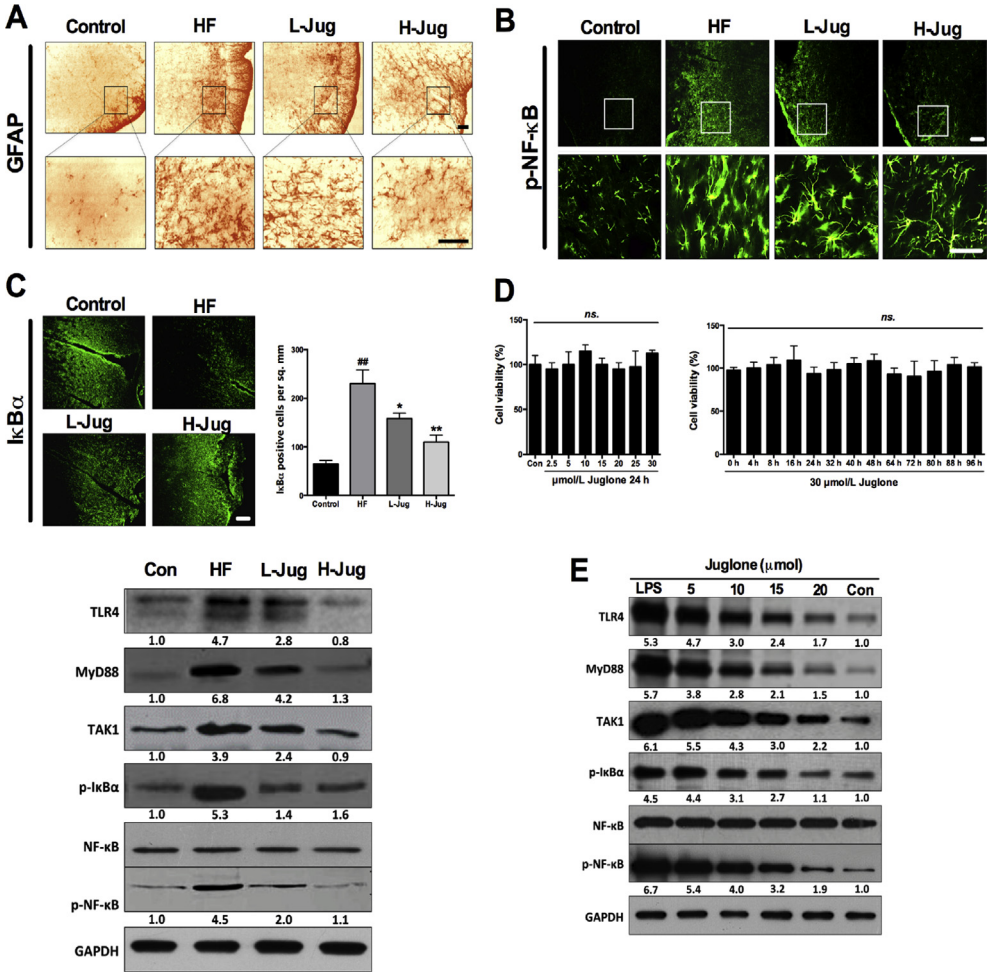

Fig. 4. Juglone inhibited astrocytes activation, NF-κB phosphorylation activation and LPS-induced primary astrocytes. (A) Immunohistochemistry analysis of brain tissue cross-cutting shows the GFAP expression level in different treated groups; the GFAP positive astrocytes per sq. mm evaluation also has been showed below. (B) Immunofluorescence analysis of hypothalamus tissues was used to investigate the inhibitory effect of juglone on NF-κB phosphorylation activation. (C) Immunofluorescence analysis of hypothalamus tissues was used to investigate the inhibitory effect of juglone on IκBα phosphorylation expression in different kinds of administrated groups. Western blots method was used to analyze the TLR4/NF-κB pathway related indicators expression in hypothalamus tissues. (D) The highest dose of 30 umol/L juglone we used to study the possible toxicity and time-dependent manner on cell viability of primary astrocytes. The different concentration of juglone (24 h) was used to test the possible toxicity and dose-dependent manner on cell viability of primary astrocytes in vitro. (E) Western blots method was used to analyze the TLR4/NF-κB pathway related indicators expression in 100 ng/ml LPS-induced primary astrocytes.

as clinically obese and well over half the population as overweight [3,4]. Data from WHO shows that at least one billion people around the world are considered overweight or obese. So, trimming the fat has become a global problem. In these reasons caused obesity, high-fat diets as the major cause to take responsibility to development obesity has been confirmed [8]. Hence, scientists around the globe are now trying to figure out the reason why high-fat diets could lead to overweight and molecular mechanisms underlying the condition. To our current knowledge, the report notes that obesity may cause potentially fatal health problems including stroke, diabetes, cardiovascular disease and even cancer [3,24–26]. Among these, the key link of high-fat intake induced obesity is associated with the action of gut microbiota-activated endotoxemia and metabolic syndrome. High fat in the animals' diet leads to obesity and may result in lipomas, lipemia, and hepatic lipidosis (NAFLD). The recent report demonstrated that HFD-induced NAFLD might enhance the development of neuroinflammation and further promote the formation of nerve injury [27,28].

Juglone, a kind of a natural active product as a food and medicine isolated from walnut husks of Juglans mandshurica, which was used to be chemoprevention of traditional food in many countries including China, India and Malaysia has been confirmed to perform anti-tumor activity [20]. For a long time, researchers pay more attention to its anti-cancer effect on various kinds of tumor. As an important source of chemoprevention in folkloric statement of many countries, we were wondering whether juglone has a protective ability to suppress endotoxemia and metabolic syndrome induced nonalcoholic fatty liver disease and nerve injury in HFD rats. Hence, in this regards, high fat diets, as an important and certified contributors to the pathological process of obesity caused by activate TLR4/NF-κB in vitro, was used to investigate whether juglone perform potent anti-inflammatory action and underlying mechanism between them.

A lot of reports have given fact that HFD-induced inflammation by releasing a number of inflammatory cytokines such as IL-1β, IL-6 and TNF-α [29]. In our present study, juglone treatment for HFD rats decreased the related indicators of hepatitis in serum and liver tissues. As expected in Table 1, juglone has ability to suppress AST, ALT, TG, TC and HDL increase during HFD period, compared with other groups. Also, juglone treated group down regulated the

expression of TNF-α, IL-1β and IL-6 in serum and rat hypothalamus. Of note, after the observation of major pro-inflammatory cytokines, the inhibition of juglone for cytokines in response to the increase of action time between 1 week and 10 week, which made us to study the dynamic changes of cytokines in serum between juglone effect and inflammatory responses. Our data showed that the expression of cytokines in HFD rats is in a time-dependent manner. Meanwhile, we also investigated that the changes of mRNA level of GFAP and CD11b in model rats. As anticipated, HFD could significantly up-regulate the mRNA level of inflammatory cytokines and glial makers.

Nuclear factor-kappa B (NF-κB), as the transcription factors that play vital roles in inflammation, immunity, cell proliferation, differentiation, and survival, has been determined to be the main signaling pathway in the development of inflammatory disease including hepatitis, nerve injury and lung injury, which is activated via Toll-like receptor (TLR) 4 to carry on regulation of inflammation [16–18]. As a matter of fact, NF-κB has been regarded as a central link in the pathogenic processes of inflammatory responses, and it may indicate a target point in treating inflammatory disease. In our data, we try to illustrate whether TLR4/NF-κB pathway is response to the inhibitory effect of juglone in HFD-induced hypothalamus inflammation. Western blots and immunofluorescent assay were used to determine, and the results declared that treatment of juglone significantly blocked the up regulation of GFAP and TLR4. Besides, juglone is capable of suppressing NF-κB activation and the degradation of IκBα. These showed that the inhibition of juglone effect on HFD induced hypothalamus inflammation might be significantly involved in the TLR4/NF-κB signaling pathway. All of the above results may provide the reason to explain the underlying mechanism of juglone inhibitory effect in liver injury and astrocytes activation stimulated hypothalamus inflammation. Besides, analysis of data indicated that TLR4/NF-κB signaling pathway as the major inflammatory pathway may be directly or indirectly involved in the development of HFD-induced inflammation.

Brief summary, we taken advantage of HFD rat model to investigated whether juglone have a potential anti-inflammatory ability to recover liver injury and hypothalamus inflammation, and the underlying molecule mechanism by which it may suppress inflammatory responses. Accumulating evidences showed that juglone significantly down regulated pro-inflammatory expression such as TNF-α, IL-1β and IL-6. Interestingly, in part of underlying mechanisms, NF-κB as the major link in the inflammation related pathway has been determined that it could be suppressed by juglone in development of hepatitis and nerve injury. Hence, juglone may perform a novel potential role or therapeutic in treating hepatitis or nerve injury, except for the known inhibitory effect of juglone on various kinds of cancer. Necessarily, in order to make the treatment more rigorous, in-depth researches and discussion on large humanoid animals and clinical investigations are confirmed to identify these findings, acquired in mice models.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Transparency document

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References

[1] A.I. Vazquez, Y.C. Klimentidis, E.J. Dhurandhar, Y.C. Veturi, P. Paerez-Rodriguez, Assessment of whole-genome regression for type II diabetes, PLoS One 10 (2015) e0123818.
[2] G.T. Russo, G. Baggio, M.C. Rossi, A. Kautzky-Willer, Type 2 diabetes and cardiovascular risk in women, Int. J. Endocrinol. 2015 (2015) 832484.
[3] J. Sargent, Obesity: obesity turns the hepatic epigenetic clock forward, Nat. Rev. Endocrinol. 11 (2015) 2.
[4] K.C. de Git, R.A. Adan, Leptin resistance in diet-induced obesity: the role of hypothalamic inflammation, Obes. Rev. 16 (2015) 207–224.
[5] H. Pan, J. Guo, Z. Su, Advances in understanding the interrelations between leptin resistance and obesity, Physiol. Behav. 130 (2014) 157–169.
[6] F. Mitchell Baleta, Country in Focus: diabetes and obesity in South Africa, Lancet Diabetes Endocrinol. 2 (2014) 687–688.
[7] K. Sohn, Sufficiently good measures of obesity: the case of a developing country, J. Biosoc. Sci. 46 (2014) 797–817.
[8] P.D. Cani, R. Bibiloni, C. Knauf, et al., Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice, Diabetes 57 (2008) 1470–1481.
[9] M.A. Hildebrandt, C. Hoffmann, S.A. Sherrill-Mix, et al., High-fat diet determines the composition of the murine gut microbiome independently of obesity, Gastroenterology 137 (2009), 1716-1724 e1711–1712.
[10] H. Daniel, A. Moghaddas Gholami, D. Berry, et al., High-fat diet alters gut microbiota physiology in mice, ISME J. 8 (2014) 295–308.
[11] T. Fujita, Is endotoxemia a therapeutic target in liver injury? Hepatology 53 (2011) 1785.
[12] Y. Guo, Y. Zhang, K. Hong, F. Luo, Q. Gu, N. Lu, A. Bai, AMPK inhibition blocks ROS-NFkappaB signaling and attenuates endotoxemia-induced liver injury, PLoS One 9 (2014) e86881.
[13] S. Sharma, P. Kesav, Bilateral facial nerve palsy: a rare association with hepatitis A, Ann. Indian Acad. Neurol. 16 (2013) 729–730.
[14] D.S. Grewal, J.L. Zeid, Isolated abducens nerve palsy following neonatal hepatitis B vaccination, J. AAPOS 18 (2014) 75–76.
[15] J.R. Caso, J.M. Pradillo, O. Hurtado, P. Lorenzo, M.A. Moro, I. Lizasoain, Toll-like receptor 4 is involved in brain damage and inflammation after experimental stroke, Circulation 115 (2007) 1599–1608.
[16] X. Zhang, H. Dong, S. Zhang, S. Lu, J. Sun, Y. Qian, Enhancement of LPS-induced microglial inflammation response via TLR4 under high glucose conditions, Cell. Physiol. Biochem. 35 (2015) 1571–1581.
[17] L.A. Velloso, F. Folli, M.J. Saad, TLR4 at the crossroads of nutrients, gut microbiota and metabolic inflammation, Endocr. Rev. (2015) er20141100.
[18] S.M. Wi, G. Moon, J. Kim, et al., TAK1-ECSIT-TRAF6 complex plays a key role in the TLR4 signal to activate NF-kappaB, J. Biol. Chem. 289 (2014) 35205–35214.
[19] S. Cosmulescu, I. Trandafir, V. Nour, Seasonal variation of the main individual phenolics and juglone in walnut (Juglans regia) leaves, Pharm. Biol. 52 (2014) 575–580.
[20] H. Xu, X. Yu, S. Qu, D. Sui, Juglone, isolated from juglans mandshurica Maxim, induces apoptosis via down-regulation of AR expression in human prostate cancer LNCaP cells, Bioorg Med. Chem. Lett. 23 (2013) 3631–3634.
[21] C. Lorenzo, S.M. Haffner, A. Stancakova, J. Kuusisto, M. Laakso, Fasting and OGTT-derived measures of insulin resistance as compared with the euglycemic-hyperinsulinemic clamp in nondiabetic Finnish offspring of type 2 diabetic individuals, J. Clin. Endocrinol. Metab. 100 (2015) 544–550.
[22] M. Canki, M.J. Potash, G. Bentsman, et al., Isolation and long-term culture of primary ocular human immunodeficiency virus type 1 isolates in primary astrocytes, J. Neurovirol 3 (1997) 10–15.
[23] H. Zhu, M.F. Chen, W.J. Yu, et al., Time-dependent changes in BDNF expression of pentylenetetrazole-induced hippocampal astrocytes in vitro, Brain Res. 1439 (2012) 1–6.
[24] Y. Kim, C.K. Kim, S. Jung, B.W. Yoon, S.H. Lee, Obesity-stroke paradox and initial neurological severity, J. Neurol. Neurosurg. Psychiatry (2014). http://dx.doi.org/10.1136/jnnp-2014-308664.
[25] J.B. Cohen, D.L. Cohen, Cardiovascular and renal effects of weight reduction in obesity and the metabolic syndrome, Curr. Hypertens. Rep. 17 (2015) 544.
[26] J.P. Vucenik, Stains, obesity and cancer risk: evidence, mechanisms, and recommendations, Ann. N. Y. Acad. Sci. 1271 (2012) 37–43.
[27] L. Bird, Gut microbiota influences liver disease, Nat. Rev. Immunol. 12 (2012) 153.
[28] D. Spector, S. Shikora, Neuro-modulation and bariatric surgery for type 2 diabetes mellitus, Int. J. Clin. Pract. Suppl. (2010) 53–58.
[29] H. Xu, G.T. Barnes, Q. Yang, et al., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, J. Clin. Invest 112 (2003) 1821–1830.