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Conjugated Linoleic Acid Ameliorates High Fructose-Induced Hyperuricemia and Renal Inflammation in Rats via NLRP3 Inflammasome and TLR4 Signaling Pathway

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Scope: Conjugated linoleic acid (CLA), a bioactive substance predominantly found in ruminant products, improves insulin resistance and exhibits anti-inflammatory activity. The chief objective of the study is to investigate the effects and potential mechanisms of CLA on high fructose-induced hyperuricemia and renal inflammation.

Methods and results: Hyperuricemia and renal inflammation are induced in rats by 10% fructose. Hyperuricemia, insulin resistance, and renal inflammation are evaluated. CLA potently ameliorates fructose-induced hyperuricemia with insulin resistance and significantly reduces the levels of inflammation factors in serum and kidney. It reverses fructose-induced upregulation of glucose transporter 9 (GLUT9) and urate transporter 1 (URAT1) in the kidney. Moreover, CLA dramatically inhibits the activation of the nucleotide-binding oligomerization domain–like receptor family pyrin domain-containing 3 (NLRP3) inflammasome. Additionally, CLA suppresses toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88) signaling activation to inhibit nuclear factor-kB (NF-kB) signaling in the kidney of fructose-fed rats.

Conclusion: CLA ameliorates hyperuricemia along with insulin resistance and renal inflammatory, which may be associated with the suppression of renal GLUT9 and URAT1 in fructose-fed rats. Its molecular mechanism may be related to the inhibition of NLRP3 inflammasome and TLR4/MyD88 signaling pathway. Therefore, CLA may be a promising candidate for preventing fructose-induced hyperuricemia and renal inflammation.

1. Introduction

Fructose, a monosaccharide naturally found in plant, fruits, and honey, is widely used in sugar-sweetened beverages.[1] However, excessive and sustained consumption of fructose is regarded as a great risk factor for metabolic abnormalities and chronic diseases such as obesity, hypertension, insulin resistance, and hyperuricemia, which may be associated with kidney diseases.[2–4] With the development of the economy, more people bear all kinds of chronic diseases in both physical pain and financial burden, which have become the primary cause of death worldwide.[5] Data show that the major chronic diseases including cardiovascular disease, cancer, and diabetes have led to 29 million deaths in the world.[6] Some of these diseases may be related to organ dysfunction and abnormal inflammation in experimental animals and humans.[7,8] Therefore, it is vital to develop effective strategies and candidates to prevent the potential damage of high fructose-induced metabolic disorders and chronic diseases in daily life.

The high uric acid level in serum is regarded as a key risk factor for renal diseases. The level of uric acid in serum is partly regulated by urate transport-related proteins. The expression of glucose transporter 9 (GLUT9) and renal transporter 1 (URAT1) are significantly increased in fructose-induced hyperuricemic mice, which is associated with inflammatory responses.[9] In the inflammation system, toll-like receptor 4 (TLR4) plays an important role in regulating a cascade of events in the body.[10] The activation of TLR4 triggers the myeloid differentiation factor 88 (MyD88) signaling pathway, which initiates the rapid activation of nuclear factor-kB (NF-kB), and increases the levels of IL-18, IL-6, tumor necrosis factor-α (TNF-α), and monocyte chemotactic protein-1 (MCP-1) both in vivo and vitro.[7,11] Moreover, tumor necrosis factor receptor-associated factor 6 (TRAF6) signalosome and TGF-β-activated kinase 1 (TAK1) are both necessary for NF-kB activation in lipopolysaccharide (LPS)-induced human cervical cancer SiHa cells.[12] Additionally, another important target factor, nucleotide-binding oligomerization domain (NOD)–like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, the member of the NOD-like receptor family, promotes innate immune defenses by the maturation of pro-inflammatory cytokines such as IL-1β and IL-18.[13] Furthermore, NLRP3 inflammasome is the complex composed of NLRP3, the adaptor

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protein apoptosis-associated speck-like protein containing a CARD (ASC), and the effector cysteine protease caspase 1 (Caspase-1).[14] Therefore, the inhibition of TLR4 signaling and NLRP3 inflammasome may provide a novel and valid approach to prevent high fructose-induced complications of renal dysfunction and inflammation in rats.

Conjugated linoleic acid (CLA), a bioactive substance predominantly found in ruminant fats, meat, and milk products, is a mixture of positional and geometric isomers with conjugated-double bonds.[15] Numerous biological benefits of the most commonly reported isomers, *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA, have been verified.[16,17] In everyday life, CLA has been used as a kind of dietary supplement owing to its diverse benefits in health promotion.[18] Previous research showed that dietary CLA could markedly improve glucose tolerance in diabetic fatty fa/fa rats.[19] It also reduced IL-1β, IL-6, and TNF-a levels in human bronchial epithelial cells through the inhibition of NF-kB signaling.[20] In addition, CLA could reduce renal damage and improve the oxidative condition in ischemia/reperfusion injury.[21] However, to the best of our knowledge, the effects of CLA on high fructose-induced hyperuricemia and renal inflammation in rats, as well as its potential mechanisms, still remain poorly characterized.

In our experiment, we investigated the effects and mechanisms of CLA on high fructose-induced hyperuricemia and renal inflammation in rats. We first confirmed whether CLA can ameliorate hyperuricemia and abnormal renal inflammation induced by 10% fructose. Additionally, we examined whether the effects of CLA are associated with the suppression of TLR4/MyD88/NF-kB signaling pathway and NLRP3 inflammasome.

2. Experimental Section

2.1. Materials

Fructose (purity 99%) was purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). CLA was supplied by Qingdao Auhai Biotech Co., Ltd (Shangdong, China), which contained 80.7% conjugated dienes (38.3% 9c, 11t-CLA, 38.1% 10t, 12c-CLA representing 94.7% of the contained isomers), 10.2% oleic acid, 2.9% stearic acid, 3.7% palmitic acid, and 1.7% linoleic acid. Uric acid and glucose assay kits were obtained from Jiancheng Bioengineering Institute (Nanjing, China). ELISA kits were obtained from Bio-Swamp Life Science (Wuhan, China). Antibodies were acquired from Proteintech Group (Chicago, USA). Goat anti-mouse IgG and goat anti-rabbit IgG conjugated to horseradish peroxide were the products of Biosharp (Shanghai, China).

2.2. Experimental Animals and Treatments

The male Sprague Dawley (SD) rats (weighing 200 g $\pm$ 20 g) were purchased from Wuhan University Laboratory Animal Center. The SD rats were housed in a drafty room, where the relative humidity was maintained between 60% and 80% with a 12 h light/dark cycle under the temperature of 22 $\pm$ 2 °C. All animal procedures were performed on the basis of the National Institute of Health Guide for the Care and Use of Laboratory Animals. In addition, all animal experiments were conducted following the guidelines on ethical use and were approved by The Institutional Animal Care and Use Committee (IACUC), Wuhan University Center for Animal Experiment, Wuhan, China (AUP No. S2017089-1).

After being acclimatized for 7 days with free access to food and drink, SD rats were randomly divided into three groups ($N = 8$): normal group, 10% fructose group, and 10% fructose + CLA (200 mg kg^{-1}) group. All rats were fed with standard pellets ad libitum for 6 weeks. The normal group received normal drinking water, while fructose and CLA groups were given drinking water with 10% fructose for 6 weeks to induce hyperuricemia, insulin resistance, and renal inflammation.[22–24] Distilled water and CLA were given orally once a day at 14:00–15:00. After 6 weeks, all rats were sacrificed. All experiments were conducted according to the procedure shown in **Figure 1**A.

2.3. Dosage Information

CLA was administered to the CLA group by gavage at the dose of 200 mg kg^{-1} once a day regularly for 6 weeks. The dose of CLA used in the experiment, which was equivalent to about 2 g per day in humans, was based on previous research.[21,25–28]

2.4. Measurement of Uric Acid in Serum and Urine

One day before sacrificing, urine was collected from the experimental rats with metabolic cages in a 24-h period. Afterward, the urine samples were centrifuged at 3000 rpm for 10 min to collect the supernatant which was diluted ten times with saline to determine the concentrations of uric acid. After the experimental rats were sacrificed, the blood samples were centrifuged at 3000 rpm for 10 min at 4 °C to collect serum samples as soon as possible. Then, the concentration of uric acid both in serum and urine was measured by using the detection kit following the commercial specification. Final results were expressed as μmol L^{-1}.

2.5. Measurement of Glucose and Insulin Resistance

When rats were maintained in a fasting condition for 8 h, the blood samples were collected to detect the concentrations of glucose and insulin in serum. These two indexes were determined by commercial kits in accordance with the standard instructions. Homeostasis model assessment of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were evaluation indexes for the resistance and sensitivity for insulin in rats.[29] The concentrations of glucose were multiplied by the concentration of insulin then divided by 22.5 to get the HOMA-IR index. Another index QUICKI can be obtained by a formula using HOMA-IR result.[30]

2.6. Histological Examination

After rats were sacrificed, sections of kidney tissue were cleaned and fixed fully in 4% paraformaldehyde for more than 24 h. To analyze the histological and morphological change of kidney,

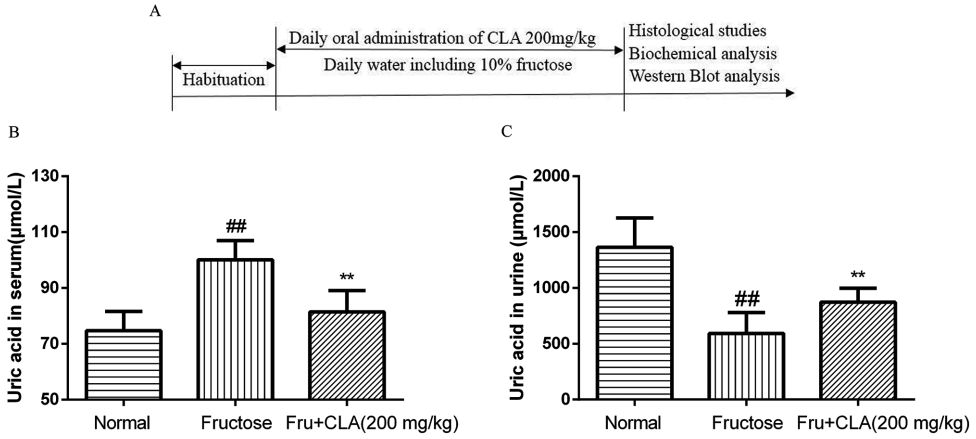

Figure 1. A) Procedure illustrating the process of the experiment including fructose and CLA administration, the time for biochemical analysis, and western blot analysis. B) Effect of CLA on the uric acid in serum; C) effect of CLA on the uric acid in urine. All the data are expressed as mean ± SD ($n = 8$). $^{\#\#}p < 0.01$ compared to the normal group; $^{**}p < 0.01$ compared to the model group.

specimens were routinely embedded in paraffin wax and sectioned into serial sections which were stained with hematoxylin-eosin (HE). Then the samples were observed under a microscope (Olympus) to collect vivid images for further analysis.

2.7. Measurement of IL-6, IL-18, IL-1β, and TNF-α in Serum and Kidney

When rats were sacrificed, kidney tissues were stored and then homogenized with pre-cooling saline solution at cold temperatures. Afterward, all the suspensions were centrifuged at 3000 rpm for 10 min at 4 °C to collect the supernatant for further biochemical detection. In the experiment, the levels of IL-6, IL-18, IL-1β, and TNF-α in serum and kidney were all carefully operated by ELISA kits following the commercial instructions, respectively.

2.8. Western Blotting

Kidney samples were homogenized in pre-cold RIPA lysis buffer with 1% phenyl-methane sulfonyl fluoride at low temperature. Afterward, the homogenate was centrifuged at 12 000 rpm for 10 min at 4 °C to gather the supernatant for western blot assay. Equal amounts of protein samples were prepared for SDS-PAGE and then transferred onto 0.45 μm polyvinylidene fluoride membranes. The membranes were blocked with 5% BSA for 30 min, then covered with the following primary antibodies at 4 °C overnight, the anti-GLUT9, anti-URAT1, anti-MCP-1, anti-NLRP3, anti-ASC, anti-MyD88, anti-TRAF6, anti-TAK1, anti-NF-κB P65 from Proteintech, and anti-Caspase1, anti-TLR4, and β-actin purchased from Santa Cruz. Later, after three washes with Tris-buffered saline-Tween solution, the membranes were covered with horseradish peroxidase-conjugated secondary antibodies at a dilution of 1:5000 for 1 h at room temperature. At last, target signals were analyzed by utilizing the chemiluminescence software to get the expression of these proteins.

2.9. Statistical Analysis

In the study, all values were given as mean ± SD. Statistical evaluation was generated using one-way analysis of variance with the SPSS 19.0 software, and graphs were conducted by Graph Pad Prism 5 software. Values of $p < 0.05$ were generally interpreted to be statistically significant.

3. Results

3.1. CLA Attenuates High Fructose-Induced Hyperuricemia in Rats

AS shown in Figure 1B, CLA administration decreased the concentration of uric acid in the serum compared to the model group from 100.1 ± 6.9 to 81.5 ± 7.6 μmol L^{-1} ($p < 0.01$). The level of CLA group was near to the normal group in serum. On the other hand, the level of uric acid in the urine was lower in the fructose group than the CLA group (591.9 ± 186.9 vs 872.5 ±125.4 μmol L^{-1}, Figure 1C, $p < 0.01$). These data demonstrated that CLA supplementation could effectively prevent high fructose-induced hyperuricemia.

3.2. Effect of CLA on Organ Indexes and Body Weight

When rats were sacrificed, different organs were collected and weighed for organ indexes (organ weight/body weight, g per 100 g), including the liver, kidney, thymus, and spleen tissue. As shown in **Table 1**, the organ indexes of kidney were similar in the three groups, whereas liver organ indexes in the fructose group increased significantly. Liver organ indexes in the CLA group decreased slightly, but there was no significant difference versus that in the fructose group. Of note, thymus and spleen organ indexes in the fructose group both showed a striking decrease versus that in the normal group, and CLA administration significantly ameliorated the phenomenon that upregulated the levels of these indexes. On the other hand, the growth rate of body

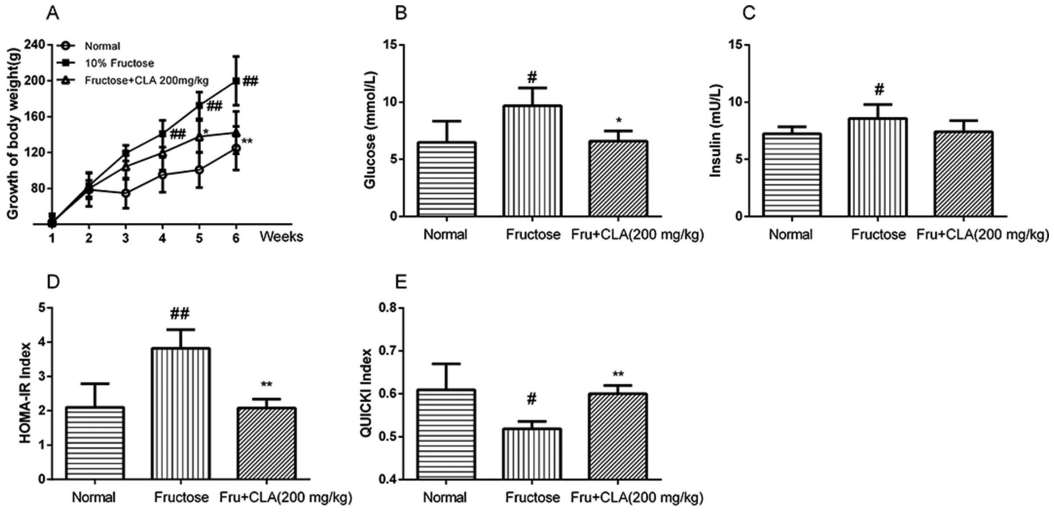

Figure 2. Effect of CLA on the growth of body weight and glucose metabolism. A) The growth of body weight; B) the level of glucose; C) the level of insulin; D) HOMA-IR and E) QUICKI were calculated to evaluate the extent of insulin resistance of the rats. All the data are expressed as mean ± SD ($n = 8$). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the model group.

Table 1. Effect of conjugated linoleic acid on organ coefficients.

	Organ index (g per 100 g body weight)			
Group	Liver [%]	Kidney [%]	Thymus [%]	Spleen [%]
Normal	2.64 ± 0.16	0.64 ± 0.04	0.17 ± 0.06	0.25 ± 0.05
Fructose	3.11 ± 0.36$^{\#}$	0.61 ± 0.08	0.10 ± 0.02$^{\#}$	0.18 ± 0.03$^{\#}$
CLA	3.00 ± 0.22	0.60 ± 0.03	0.14 ± 0.04*	0.21 ± 0.02*

Data are expressed as mean ± SD ($n = 8$); $^{\#}p < 0.05$ compared to the normal group; $^{*}p < 0.05$ compared to the model group.

weight in the model group was obviously the fastest and the supplementation of CLA showed a slowdown in the phenomenon from 4 to 6 weeks in **Figure 2**A.

3.3. CLA Improves the Insulin Resistance in Rats Fed by High Fructose

Figure 2B showed hyperglycemia happened in the 10% fructose group and the CLA significantly decreased the concentration of glucose (9.68 ± 1.56 vs 6.58 ± 0.90 mmol L^{-1}, $p < 0.5$). Furthermore, CLA restored fructose-induced increase of insulin in serum to some extent (Figure 2C). Additionally, HOMA-IR and QUICKI were acquired for the evaluation of insulin sensitivity in the experimental rats. The sensitivity of insulin maintained better in the CLA administration group than the 10% fructose group as shown in Figure 2D ($p < 0.05$), which was confirmed again by the QUICKI index in Figure 2E.

3.4. CLA Protects Against the Injury of the Kidney

H&E staining (X200) showed normal renal architecture in the kidney tissue from the normal group as indicated in **Figure 3**. The renal of the model group showed tubular edema and inflammatory cell infiltration compared with the normal group, which was signed by the black arrows (Figure 3). In addition, CLA administration robustly alleviated these tubulointerstitial pathologies induced by 10% fructose. All these results of H&E staining demonstrated that CLA could ameliorate the damage of kidney including abnormal inflammation and the tubular edema in 10% fructose-fed rats.

3.5. CLA Reverses the Alteration of Renal GLUT9 and URAT1 in 10% Fructose-Fed Rats

Literature shows that increased expressions of GLUT9 and URAT1 were observed in the kidney of fructose-fed rats.[9] In this study, the levels of renal GLUT9 and URAT1 were markedly increased in 10% fructose group compared with the normal group (**Figure 4**B, $p < 0.01$). Moreover, the expression of GLUT9 and URAT1 were downregulated in the CLA group compared with the fructose group.

3.6. CLA Relieves the Systemic Inflammation in Serum and Kidney of High Fructose-Fed Rats

We investigated whether CLA affected inflammatory responses in serum and kidney of fructose-induced hyperuricemic rats. Representative inflammatory cytokines were measured following respective instructions by ELISA kits including IL-6, IL-18, IL-1β, and TNF-α in the kidney, which play an important role in many kinds of diseases.[31] As shown in **Figure 5**A–H, 10% fructose could markedly increase the levels of IL-1β, IL-18, IL-6, and TNF-α both in serum and kidney, then CLA administration could significantly reduce the concentrations of all these inflammatory biomarkers versus the model group. Moreover, MCP-1 is one of the vital chemokines which participate in the development of

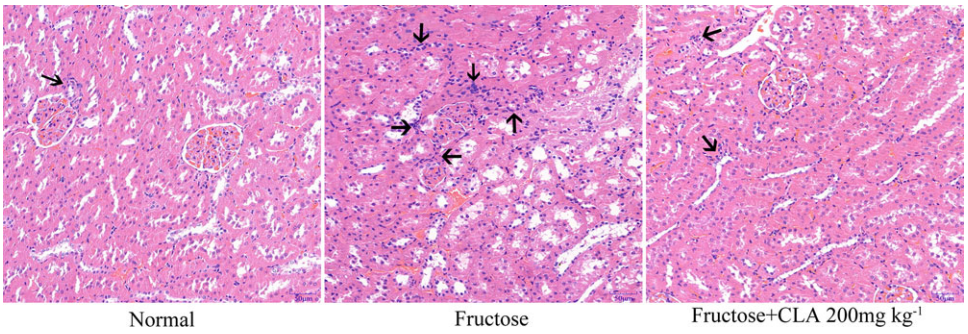

Figure 3. Effect of CLA administration on the pathological injury in the kidney of high fructose-fed rats showed by H&E staining of the kidney, magnification, X200, scale bar = 50 μm. The obvious damage was marked by the arrows.

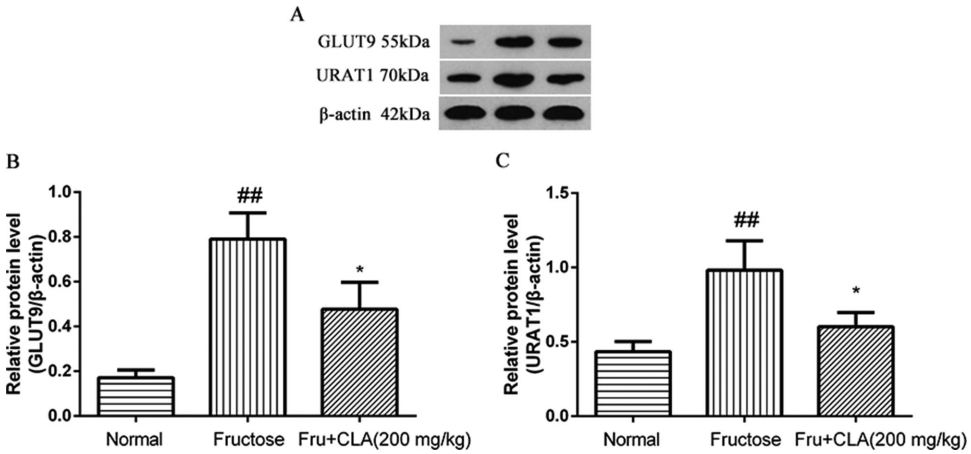

Figure 4. Effect of CLA on protein levels of GLUT9 and URAT1 in the kidney of high fructose-fed rats. A) Western blot analysis for GLUT9 and URAT1 in the kidney. Protein relative levels of B) GLUT9 and C) URAT1 were normalized to β-actin. All the data are expressed as mean ± SD ($n = 8$). $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$ compared to the model group.

migration/infiltration of monocytes and macrophages.[32] In this respect, fructose increased the expression of MCP-1 protein in kidney tissue compared with the normal group, then CLA could downregulate the growing trend versus that in the model group as shown in **Figure 6**B ($p < 0.05$). From these data, we can draw a conclusion that CLA could alleviate the systemic inflammation in serum and kidney induced by 10% fructose.

3.7. CLA Inhibits the NLRP3-ASC-Caspase1 Inflammasome in Kidney

To investigate whether NLRP3 inflammasome plays an important role in the experiment, closely relevant proteins were researched and analyzed by western blot analyses, including NLRP3, ASC, and Caspase-1. In the result, 10% fructose group showed the striking elevation in NLRP3, ASC, and Caspase-1 proteins compared with the normal group. Furthermore, the administration of CLA remarkably suppressed the expression of all those target proteins versus that in the model group (Figure 6C–E, $p < 0.05$). These results made it clear that CLA may inhibit the NLRP3-ASC-Caspase1 pathway to produce some physiological effect.

3.8. CLA Suppresses TLR4 and MyD88 Signaling in the Kidney of Rats

TLR4 plays an important role in innate immunity as described before. In this experiment, the expression of TLR4 was significantly upregulated compared with the control group as shown in **Figure 7**B ($p < 0.01$), then CLA inhibited the activation of TLR4 protein versus the trend in the 10% fructose group (Figure 7B, $p < 0.05$). On a deep level, research indicates that MyD88 is involved in signal transduction for most TLRs such as TLR4.[33] Therefore, MyD88 protein was listed as the target in the research and was found prominently inhibited by CLA compared with the 10% fructose group in Figure 7C ($p < 0.05$). These results suggested that CLA could significantly inhibit the activity of TLR4 and MyD88 in the kidney of rats fed by high fructose.

3.9. 3.8 CLA Potently Inhibited the Expression of TRAF6, TAK1, and NF-kB Proteins

In the downstream of the MyD88 signaling pathway, there are various kinds of proteins such as TRAF6, TAK1, and NF-kB.[34,35] **Figure 8**B,C showed that 10% fructose administration notably

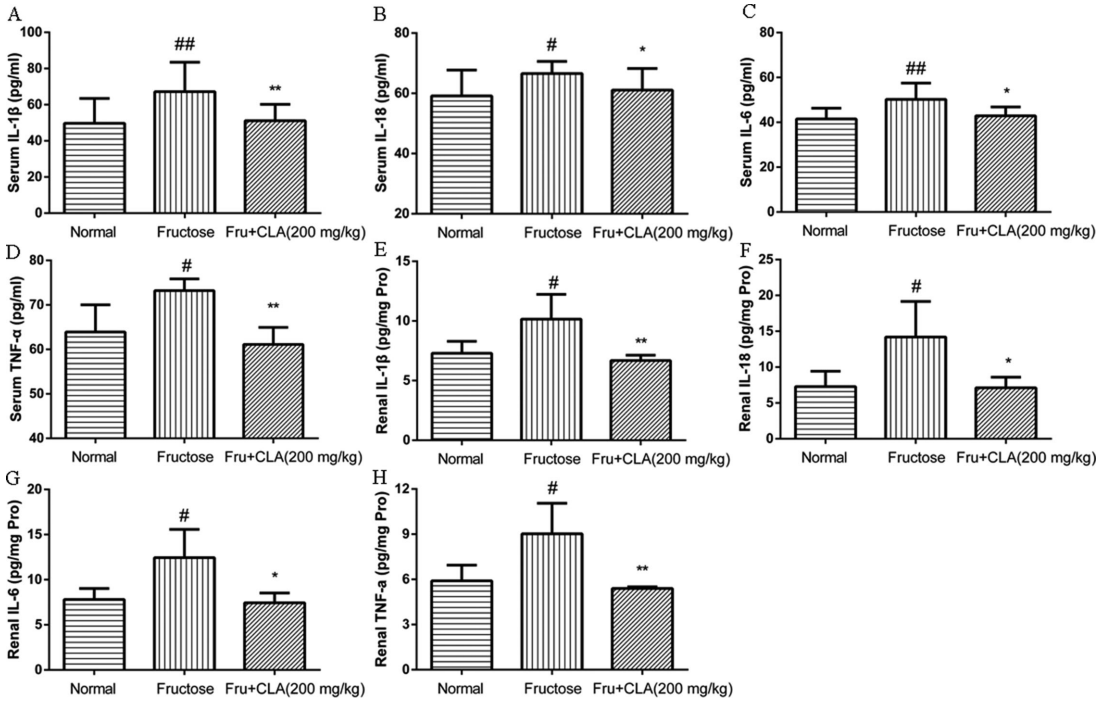

Figure 5. Effects of CLA on inflammatory cytokine levels in serum and kidney of high fructose-fed rats. Serum levels of A) IL-1β, B) IL-18, C) IL-6, and D) TNF-α, as well as kidney levels of E) IL-1β, F) IL-18, G) IL-6, and H) TNF-α, were measured by ELISA kits, respectively. All the data are expressed as mean ± SD ($n = 8$). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to the model group.

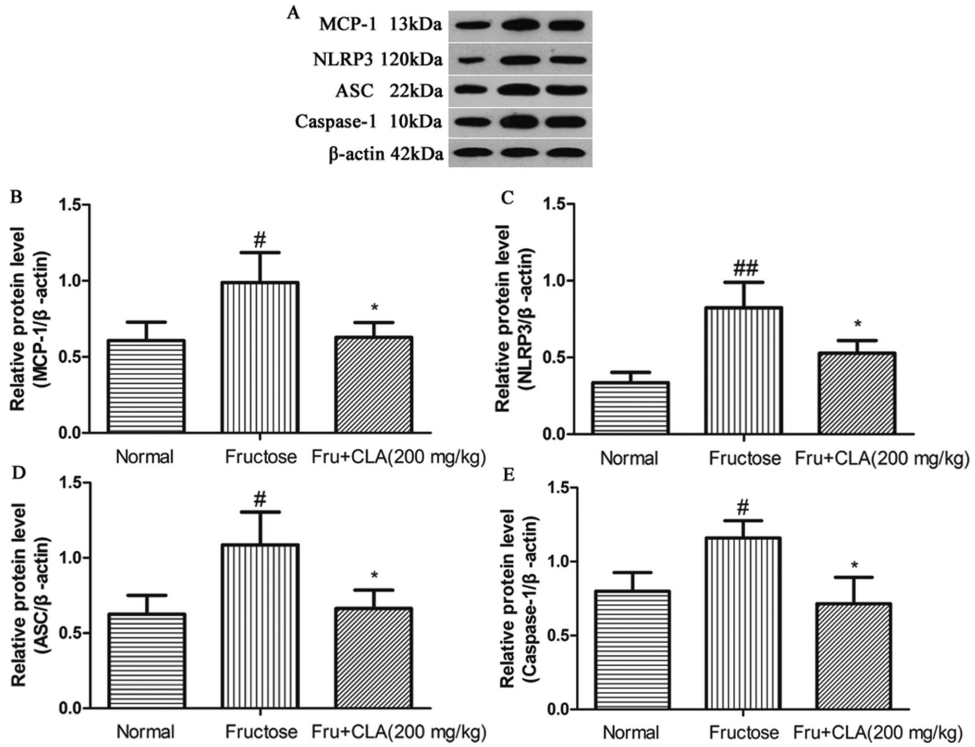

Figure 6. Effect of CLA on protein levels of MCP-1, NLRP3, ASC, and Caspase-1 in the kidney of high fructose-fed rats. A) Western blot analysis for MCP-1, NLRP3, ASC, and Caspase-1 in the kidney. Protein relative levels of B) MCP-1, C) NLRP3, D) ASC, and E) Caspase-1 were normalized to β-actin. All the data are expressed as mean ± SD ($n = 8$). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$ compared to the model group.

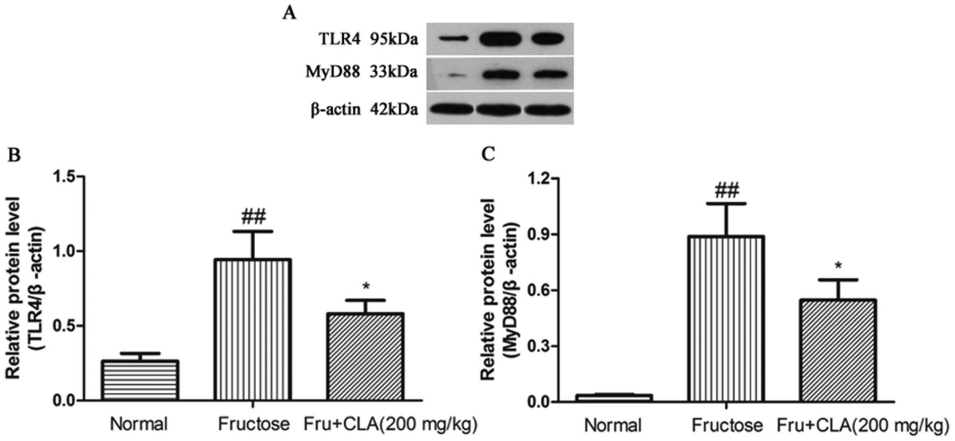

Figure 7. Effect of CLA on protein levels of TLR4 and MyD88 in the kidney of high fructose-fed rats. A) Western blot analysis for TLR4 and MyD88 in the kidney. Protein relative levels of B) TLR4 and C) MyD88 were normalized to β-actin. All the data are expressed as mean ± SD ($n = 8$). $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$ compared to the model group.

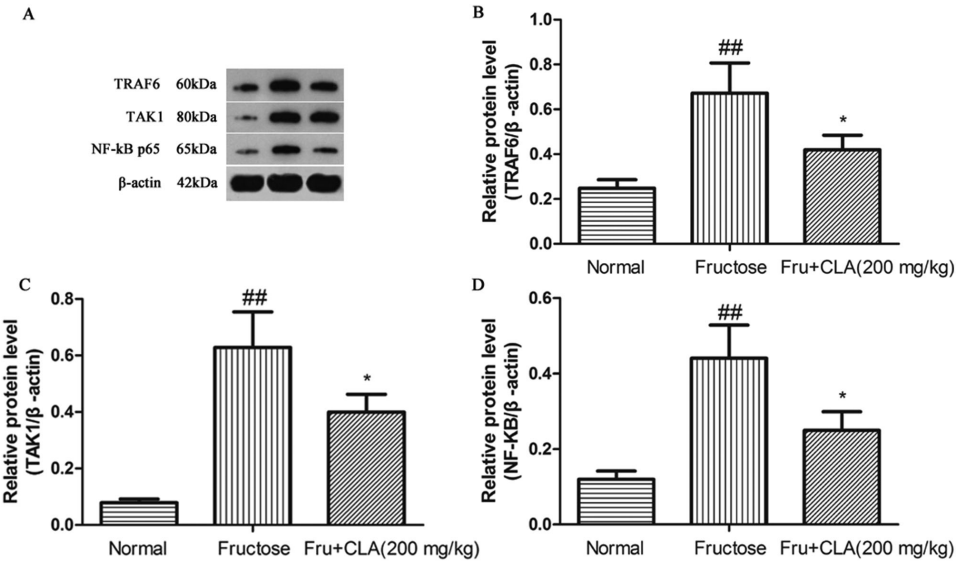

Figure 8. Effects of CLA on protein levels of TRAF6, TAK1, and NF-kB in the kidney of high fructose-fed rats. A) Western blot analysis for TRAF6, TAK1, and NF-kB in the kidney. Protein relative levels of B) TRAF6, C) TAK1, and D) NF-kB were normalized to β-actin. All the data are expressed as mean ± SD ($n = 8$). $^{\#\#}p < 0.01$ compared to the normal group; $^{*}p < 0.05$ compared to the model group.

upregulated the expression of TRAF6 and TAK1 in the kidney ($p < 0.01$). However, CLA owned the lower expression of both TRAF6 and TAK1 proteins versus the model group ($p < 0.05$). Above all, the NF-kB protein also presented the same trend that the fructose motivated the level of NF-kB expression compared with the normal group (Figure 8D, $p < 0.01$) and CLA could downregulate the expression versus the fructose group (Figure 8D, $p < 0.05$). These results showed that CLA significantly inhibited the expression of TRAF6, TAK1, and NF-kB in the kidney.

4. Discussion

In the experiment, we demonstrated that: 1) CLA prevents 10% fructose-induced hyperuricemia with insulin resistance in experimental rats; 2) CLA significantly attenuates the levels of inflammatory factors in serum and kidney; 3) CLA may regulate renal GLUT9 and URAT1 to ameliorate fructose-induced hyperuricemia; 4) the effect of CLA is related to the inhibition of NLRP3 inflammasome and TLR4/MyD88/NF-kB signaling pathway.

As a sweetener, fructose has become a part of human daily life. According to a meta-analysis, fructose consumption is an important risk factor for some chronic disorders such as metabolic syndrome in healthy adults.[36] Increasing in vivo and in vitro studies demonstrate that overconsumption of fructose causes hyperuricemia, insulin resistance, inflammation, and organ dysfunction.[7,24,37] CLA is reported to improve insulin resistance and modify the immune response to prevent immune-induced wasting.[38] Thymus and spleen are the primary immune system organs. In the present study, CLA also improved insulin resistance and partly restored thymus and spleen organ indexes

in high fructose-fed rats. The reduction of thymus and spleen organ indexes may indicate that inflammation occurred in rats. Moreover, CLA significantly reduced the concentration of uric acid in serum. Of note, CLA decreased the expression of renal GLUT9 and URAT1 in the fructose-induced hyperuricemia rats. To our knowledge, no one has reported that CLA could ameliorate hyperuricemia and regulate urate transport-related proteins in fructose-fed rats. The effects of CLA to regulate renal GLUT9 and URAT1 may be related to the inflammatory response. Further study is needed to investigate whether CLA directly regulates the transcription of GLUT9, URAT1, and other urate transport-related proteins.

A number of researches demonstrate that a high level of uric acid in serum is closely related to inflammatory response and renal dysfunction.[7,9,39] In fact, the excessive consumption of high fructose increases the levels of IL-1β, IL-6, and TNF-α in serum and kidney of fructose-fed rats, which lead to kidney dysfunction. Moreover, the production of MCP-1, which is a positive correlation with kidney injury, increases in fructose-exposed HK-2 cells.[7] It is well known that patients with gout often take anti-inflammatory drugs to relieve symptom slightly in daily life.[40] In addition, c-9,t-11 CLA decreases the production of IL-6, TNF-α, and MCP-1 in adipocytes by inhibiting the activation of NF-kB signaling.[41] In the present study, CLA was found to reduce IL-1β, IL-18, IL-6, TNF-α, and MCP-1 levels in the kidney of fructose-induced hyperuricemia rats.

Both NLRP3 inflammasome and TLR4 signaling play a pivotal role in kidney injury with hyperuricemia. NLRP3 inflammasome is the polyprotein complex composed of innate immune receptor NLRP3, adaptor ASC, and protease Caspase-1.[42,43] In addition, TLR4 plays a role as a promoter in the inflammatory response chain in the body.[44] The inhibition of NLRP3 and TLR4 signaling pathway is a promising potential target in metabolic diseases.[45,46] Moreover, there is no report that the anti-inflammation effect of CLA on fructose-induced hyperuricemia rats by inhibiting NLRP3 inflammasome and TLR4 signaling pathway. In this manuscript, CLA decreased the expression of NLRP3/ASC/Caspase-1 and inhibited the TLR4 signaling pathway, and then downregulated downstream proteins including MyD88, TRAF6, TAK1, and NF-kB proteins. The suppression of NLRP3 inflammasome and TLR4 signaling decreases inflammatory response, which may regulate the expression of GLUT9 and URAT1 to reduce the uric acid level.

In conclusion, we confirm that CLA administration can ameliorate hyperuricemia and renal inflammation in high fructose-fed rats. CLA decreases the inflammation level to protect the kidney and the function of CLA may be associated with the inhibition of NLRP3 and TLR4 signaling pathway. The experiment proved that CLA could be one of the safe and effective candidates to ameliorate hyperuricemia and renal inflammation in daily life. Furthermore, whether the anti-inflammation efficacy of CLA makes a difference in other specific metabolic diseases needs more research to confirm for wider application.

Acknowledgements

J.Y.T. and L.P.W. contributed equally to this work. H.D. contributed to conceive and design the study. J.Y.T., L.P.W., X.F.C., X.L.L., X.X.H., X.F.L., and J.L. conducted the animal experiment. J.Y.T., L.P.W., and X.F.C. participated in the WB experiment. J.Y.T. and L.P.W. contributed to data analysis and manuscript drafting. H.D. contributed to critical manuscript revision. All authors had checked and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

conjugated linoleic acid, hyperuricemia, fructose, renal inflammation, toll-like receptor 4

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