Effect and Mechanism of Lycium Barbarum Polysaccharide on Hyperglycemia and Inflammation in Diabetic KKAy Mice

hong yin
Yangzhou University https://orcid.org/0000-0002-2973-3801
Shuying Deng
Ningxia Medical University
Lili Bai
Ningxia Medical University
Lihua Li
lianshui county hospital
Tingting Liu
Ningxia Medical University
Huizhen Cai (18169010183@163.com)
https://orcid.org/0000-0002-2507-3631

Research

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Abstract

Scope The aim of this study is to examine whether lycium barbarum polysaccharide (LBP) supplementation improves hyperglycemia and inflammation in diabetic KKAy mice.

Methods The successfully established diabetic KKAy mice are randomized into five groups: diabetic model, metformin, low-dose LBP, middle-dose LBP, and high-dose LBP . C57BL/6J mice are fed a chow diet as normal control . The blood glucose and body weight of mice were measured at different time points. At the end of 90 days, serum inflammatory factors were determined with ELISA kits. The expression of TLR4, MyD88, TRAF6, IκKβ, IκB, P-IκB and nuclear NF-κB proteins in mouse peritoneal macrophages were detected by Western Blotting.

Results Blood glucose decreased significantly after the intervention among low-, medium-dose LBP groups and Met group (P <0.05). Met (40 mg/kg) inhibited the levels of IL-1β, TNF-α and IL-6 and elevated IL-10 level (P <0.05). ELISA results showed that LBP promoted serum levels of IL-10 and decreased TNF-α level (P <0.05). Compared with model group, KKAy mice in Met group expressed lower protein levels of MyD88, TRAF6, IκKβ, nuclear NF-κB and higher expression of IκB (P <0.05); The expression of TLR4, MyD88, TRAF6, IκKβ and nuclear NF-κB protein in low- and medium-doses LBP groups were significantly declined (P <0.05).

Conclusion These findings indicate that dietary supplementation with LBP can improve hyperglycemia and inflammation in diabetic KKAy mice, which can be associated with potential benefits to human health.

Introduction

The global report on diabetes from WHO clarifies that in 2014, 422 million people in the world had diabetes with a prevalence of 8.5% among adult population. The prevalence in China was higher than the global average prevalence, total adult prevalence of 9.4% with most diabetic patients in the world [].
Compared to non-diabetic patients, diabetes patients lead to complications, such as blindness, kidney failure, low limb amputation and so on, which consequently, significantly impact on quality of life. Recent studies have shown that diabetes mellitus and its complications are closely related to inflammation.

The fruit of *Lycium barbarum* (Goji berry) has been commonly used as traditional Chinese medicine and herbal food for health promotion in countries. Goji berry is mainly comprised of polysaccharides, carotenoids, vitamin C, flavonoids, essential oil, fatty acids, glycerogalactolipids, free amino acids and miscellaneous compounds. Among these constituents, *Lycium barbarum*polysaccharides (LBP) has been most widely researched and considered to be the main bioactive substance. The monosaccharide composition of LBP contained rhamnose, arabinose, xylose, mannose, glucose, galactose, galacturonic acid [].

Shi J et al's results [] demonstrate that LBP exerts protective effects on diabetes induced male spermatogenic dysfunction, which is likely to be mediated through increasing antioxidant enzyme activities and inhibiting cell death. The study of Xia H et al [] exhibited the effects of LBP on the urine and liver metabolomics in a high-fat diet and streptozotocin-induced diabetic rat model. LBP improves cardiac hypertrophy, inhibits the expression of calpain-1 and inhibits the activation of NF-κB in diabetic rats [].

Few clinical studies investigated the protection of LBP on postprandial glucose and lipid metabolism in type 2 diabetic patients [ ]. And LBP reduces vascular lesions induced by T2DM through regulating p38MAPK signaling pathways and increasing antioxidative capacity [].

In a word, the beneficial effects of LBP on diabetes have been confirmed by animal and clinical studies. However, the effect of LBP on inflammation is unclear, which may provide the new evidence of protection mechanism of LBP on diabetes. Therefore, in the present study we attempted to explore the effects of LBP on hyperglycemia and inflammation in diabetic KKAy mice.

Materials And Methods

Preparation of LBP

LBP was prepared as described previously []. Dried *L. barbarum* was made into a powder and decocted with water (60 °C) by a traditional method used for Chinese medicinal herbs after degreasing. Then it was filtered by regenerated cellulose membranes of 300 kDa, 100 kDa, 80 kDa, 50 kDa and 30 kDa (0.2 MPa, 60 °C) after centrifuging. The resulting fraction was retained and vacuum-dried at 40 °C. Neutral sugars were determined by phenol-H_2SO_4, acidic sugars by carbazole

and proteins by the Coomassie Brilliant Blue G-250 method .

LBP we prepared was a brown powder composed of neutral sugars (78.23%) and acidic sugars (14.83%). The protein content was < 6.92%.

Animals and Treatment

Five weeks old spontaneously diabetic female KKAy mice and age-matched female nondiabetic C57BL/6J mice were obtained from Beijing HFK Bioscience Co., Ltd (Beijing, China). All mice were housed one per cage at 22 ± 2 °C with a 12-h light/dark cycle. The KKAy mice were fed a high fat diet, which has applied for national invention patent of China (application number: CN201110127312.5). The C57BL/6J mice (n = 10) were fed normal chow die as normal control (NC). The composition of the diets is shown in Table 1. After 5 weeks of high fat diet feeding when the fasting blood glucose was higher than 16.7 mmol L^{-1}, the KKAy mice were randomly divided into five groups (n = 10 in each group): 1) diabetic model (DM); 2) Metformin group(Met), 40 mg/kg, intragastric administration ;3) low dose LBP (L),20 mg/kg, intragastric administration ;4) middle dose LBP (M), 40 mg/kg, intragastric administration ; and 5) high

dose LBP (H), 80 mg/kg, intragastric administration. NC and DM were gavaged with distilled water. The animal treatment lasted for 90 days, during which both diet and water were consumed ad libitum. At the end of experiment, overnight fasted mice were sacrificed. Blood and tissue samples were collected for further analysis. The animal experimental protocols were conducted according to the Institutional Animal Care and Use Committee of Ningxia Medical University.

Table 1
Composition of the diets (per 100 g)

	High-fat diet	Chow diet
Carbohydrate [g]	48.5	52
Protein [g]	17.5	18
Fat [g]	17.9	4
Energy from carbohydrate [%]	37.9	65.8
Energy from protein [%]	16.5	22.8
Energy from fat [%]	45.6	11.4

Collection of macrophages from mouse abdominal cavity

The mice were sacrificed and immersed in 75% ethanol solution for 2–3 minutes to disinfect the skin. Inject incompletely 1640 culture solution into the abdominal cavity of mice in sterile environment with 1 ml syringe needle, gently squeeze the abdominal wall with hand for more than 20 times, and suck out the irrigation solution. Take a small amount of cell suspension for Giemsa staining, and observe under the light microscope to identify whether it is macrophage. The supernatant was removed after centrifugation for 2000r / min and 5 min, and then washed with Hanks for three times. RPMI1640 medium containing 15% calf serum was used to suspend the cells. The collected cells were inoculated into the cell culture dish. Cultured in a 5% CO2 incubator at 37 ℃ for 24 hours to make macrophages adhere to the wall, take out the culture dish, discard it and wash it with PBS to remove the non adherent cells, so as to obtain a relatively pure macrophage.

Assays of IL-6, IL-1β, TGF-β1, IL-10, IL-8 and TNF-α in Serum samples

IL-6, IL-1β, TGF-β1, IL-10, IL-8 and TNF-α were determined using a commercial ELISA kit (eBioscience company, USA), according to the instructions.

Total and nuclear protein extraction

For TLR4, MyD88, TRAF6, I κ K β, I κ B, P-I κ B and NF - κ B analysis, protein expression by western blotting, total and nuclear protein extracts were prepared from pure macrophage using commercial kits (Biosynthesis Biotechnology Co., LTD, Beijing, China). The protein concentration was determined by bicinchoninic acid (BCA) assay and stored at − 80 °C until analyzed.

Western blotting

Sixty (60) μg of cell extract were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). The membrane was blocked for 1 h with 5% non fat milk in TBST and then incubated with a rabbit monoyclonal antibody against TLR4 (AbcamCompany, UK) at 4 °C over night. After washing with TBST, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary anti-rabbit antibodies (1:5000; Boster Co., Wuhan, China) for 60 min at room temperature. After additional washing, bound conjugates were detected by ECL superSignalTM West Pico substrate (Pierce, Rockford, IL, USA). Proteins were visualized by exposing the blot to X-ray film, photographed with a digital camera, and then the net intensities of the individual bands were measured using Bandscan 5.0 software. Rabbit anti-β-Actin monoclonal antibody (AbcamCompany, UK) was used as the loading control, and TLR4 protein expression was normalized to Actin.

The WB process of MyD88, TRAF6, I κ K β, I κ B, P-I κ B and NF - κ B are the same as that of TLR4.

Statistical analysis

Data were analyzed statistically using SPSS 16.0 for Windows and expressed as the mean ± SD of 10 mice per group. Experimental results were compared by one-way ANOVA with least significant difference (LSD) post-hoc tests used to compare individual means as appropriate. $P < 0.05$ or $P < 0.01$ were considered statistically significant.

Results

The weight changes of T2DM mice after LBP intervention

As can be seen in Table 2, the weight of mice in each group increased significantly compared with normal control ($P < 0.01$). During the experiment, the weight of mice in each group increased significantly ($P < 0.01$), but during and after the intervention, there was no significant difference between the diabetic model group and other intervention groups ($P > 0.05$).

Table 2
Effect of LBP on body weight of T2DM mice (g)

Group	0	10d	20d	45d	90d
NC	18.48 ± 0.52	19.02 ± 0.69	20.14 ± 0.39	19.97 ± 0.62	22.1 ± 0.61
Met	31.32 ± 3.14[a]	38.47 ± 3.01[a]	40.36 ± 3.60[a]	40.73 ± 3.7[a]	52.24 ± 2.41[a]
DM	31.17 ± 2.32[a]	38.38 ± 0.92[a]	41.05 ± 1.27[a]	43.43 ± 1.90[a]	52.09 ± 3.22[a]
L	32.01 ± 3.52[a]	38.29 ± 3.30[a]	40.26 ± 3.59[a]	43.72 ± 3.85[a]	52.53 ± 4.16[a]
M	33.42 ± 5.37[a]	39.56 ± 5.09[a]	39.92 ± 4.98[a]	43.21 ± 4.78[a]	50.89 ± 4.10[a]
H	33.28 ± 2.86[a]	38.31 ± 4.34[a]	39.83 ± 4.85[a]	43.58 ± 4.38[a]	50.25 ± 3.62[a]

Data are expressed as mean ± SD.
NC, normal control; Met, Metformin group; DM, diabetic model; L, low dose LBP; M, middle dose LBP; H, high dose LBP
P^{a} <0.01 versus NC
P^{b} <0.05 versus DM
P^{c} <0.01 versus Met.

Changes of blood glucose in T2DM mice after LBP intervention

Table 3 shows that before the intervention, there was no statistical difference in blood glucose between the diabetic model group, metformin group and LBP low, medium and high dose groups (P > 0.05), and the difference was significant compared with normal control (P < 0.05). After 45 days of intervention, the blood glucose in the diabetic model group was significantly higher than that in metformin group and LBP low, medium and high dose group (P < 0.05). At the end of the intervention, there was a statistical difference between LBP low and middle dose group and diabitc model group (P < 0.05), no statistical difference between LBP high dose group and diabitc model group (P > 0.05), and there was a statistical difference between LBP high dose group and metformin group (P < 0.05).

Table 3
Effect of LBP on blood glucose in T2DM mice (mmol/L)

Group	0	45d	90d
NC	7.66 ± 1.24	8.30 ± 0.56	7.16 ± 0.69
Met	17.29 ± 9.01[a]	16.99 ± 2.31[ab]	12.86 ± 4.85[ab]
DM	18.76 ± 3.26[a]	22.14 ± 4.13[a]	22.51 ± 3.04[a]
L	19.89 ± 5.26[a]	17.19 ± 3.95[ab]	17.32 ± 4.90[ab]
M	18.07 ± 4.84[a]	16.39 ± 4.02[ab]	16.96 ± 5.30[ab]
H	18.67 ± 3.98[a]	18.27 ± 3.97[ab]	20.33 ± 3.89[ac]

Data are expressed as mean ± SD.
NC, normal control; Met, Metformin group; DM, diabetic model; L, low dose LBP; M, middle dose LBP; H, high dose LBP
P^{a} <0.01 versus NC
P^{b} <0.05 versus DM
P^{c} <0.01 versus Met.

Changes of serum inflammatory factors in T2DM mice after LBP intervention

It can be seen from Table 4 that compared with normal control, the levels of IL-1 β, IL-6, TNF - α in the serum of the diabetic model group increased (P < 0.05), and the levels of IL-10 and TGF - β 1 decreased (P < 0.05); the levels of IL-8 increased, but the difference was not statistically significant (P > 0.05). Compared with the diabetic model group, the levels of IL-1 β, IL-6, TNF - α decreased, IL-10 increased, TGF - β 1 and IL-8 had no statistical difference (P > 0.05), which indicated that metformin could effectively change the inflammatory state of T2DM model. After LBP intervention, compared with the diabetic model group, three doses of LBP can effectively increase IL-10 (P < 0.05) and reduce TNF - α level (P < 0.05); low doses of LBP can also significantly inhibit the increase of IL-1 β level (P < 0.05); and these changes have no statistical difference compared with the metformin group (P > 0.05), which shows that LBP can effectively change the levels of IL-10, TNF - α and IL-1 β in serum, and its action Compared with metformin, there was no significant difference (P > 0.05).

Table 4

Effect of LBP on serum inflammatory factors in T2DM mice (pg/mL)

Group	IL-1β	IL-6	IL-8	IL-10	TNF-α	TGF-β1
NC	34.66 ± 18.22^{b}	12.93 ± 7.98^{b}	225.62 ± 174.41	3.893 ± 0.935^{b}	38.48 ± 1.19^{b}	143.05 ± 21.28 ^{bc}
Met	36.52 ± 10.97^{b}	18.67 ± 12.96^{b}	260.47 ± 73.85	4.519 ± 1.455^{b}	39.98 ± 3.66^{b}	102.25 ± 33.12 ^{a}
DM	74.28 ± 40.12^{ac}	35.33 ± 15.09^{ac}	309.82 ± 153.36	1.350 ± 0.280^{ac}	45.19 ± 3.30^{ac}	98.41 ± 19.83^{a}
L	39.52 ± 13.36^{b}	31.13 ± 22.88^{a}	329.11 ± 121.63	4.802 ± 0.874^{b}	40.31 ± 3.35^{b}	116.31 ± 50.63
M	51.30 ± 41.13	37.85 ± 26.59^{a}	232.38 ± 33.20	4.830 ± 1.409^{b}	40.51 ± 2.28^{b}	103.45 ± 34.35 ^{a}
H	68.95 ± 39.35	24.69 ± 7.46	216.47 ± 81.05	8.671 ± 3.932^{b}	39.32 ± 1.36^{b}	113.46 ± 25.44 ^{a}
• Data are expressed as mean ± SD.						
• NC, normal control; Met, Metformin group; DM, diabetic model; L, low dose LBP; M, middle dose LBP; H, high dose LBP						
• P^{a}<0.05 versus NC						
• P^{b}<0.05 versus DM						
• P^{c}<0.05 versus Met						

Effects of LBP on TLR4, MyD88, TRAF6, I κ K β, I κ B, P-I κ B and NF - κ B protein expression in peritoneal macrophages of T2DM mice

As shown in Fig. 1, compared with NC, the expression of TLR4, MyD88, TRAF6, I κ K β, P-I κ B in cytoplasm and NF - κ B in nucleus increased ($P < 0.05$) and I κ B in cytoplasm decreased ($P < 0.05$) in diabetic model group.

After the intervention, compared with the model group, the levels of MyD88, TRAF6, I κ K β and NF - κ B in the nucleus of metformin group decreased, the expression of I κ B in the cytoplasm increased ($P < 0.05$), TLR4 decreased, but the difference was not statistically significant ($P > 0.05$).

After LBP intervention, the expression of TLR4, MyD88, TRAF6, I κ K β and NF - κ B in the nucleus decreased significantly ($P < 0.05$). The expression of MyD88 and TRAF6 was even lower than that of metformin group ($P < 0.05$). The expression of I κ B in LBP group was higher than that of diabetic model group and metformin group ($P < 0.05$). The effect of LBP on TLR4, MyD88, TRAF6 and I κ K β protein was dose-dependent. The lower the concentration of LBP, the more obvious the inhibition. However,the effect of middle dose LBP was the most significant of the effect on I κ B in cytoplasm and NF - κ B in nucleus.

Discussion

In this study, the body weight and blood sugar of KKAy mice were significantly increased compared with normal control after high-fat feed, showing the characteristics of abdominal obesity and hyperglycemia. This is consistent with the experimental results of Liu min []. Previous studies have shown that LBP can reduce body weight by increasing the hypothalamic leptin level, reducing appetite and accelerating fat metabolism in SD obese rats. But after LBP intervention, the weight of KKAy mice did not change significantly. This is not consistent with the result that LBP can reduce the body weight of obese SD rats. This may be related to the functional defect of melanocortin receptor-4 (MC4R) in the brain of KKAy mice. MC4R is a kind of peptide secreted by hypothalamus. It can affect the function of leptin by binding with melanocortin (MC), agouti protein and agouti related protein (AgRP). It plays an important role in regulating the energy balance of the body [ ]. In KKAy mice, agouti protein encoded by ay gene will bind to melanocortin receptor MC4R to antagonize the effect of leptin []. Therefore, the effect of LBP on the body weight of KKAy mice was not observed.

In this study, after the intervention of LBP, the blood glucose of mice in the low, medium and high dose groups was significantly lower than that in the diabetic model group, which was consistent with the research results of Zhao R. et al [], further verifying the hypoglycemic effect of LBP. At present, it is believed that the hypoglycemic effect of LBP is not only related to the up regulation of glucokinase (GK) and pyruvate kinase (PK) expression, but also related to the up regulation of GLUT4 and the increase of glucose uptake and utilization [ ]. Metformin can improve hyperglycemia by inhibiting gluconeogenesis of liver and increasing glucose intake of muscle. Its molecular mechanism is related to activation of adenylate activated protein kinase, protein kinase A and inhibition of mitochondrial respiratory enzyme [,]. The results of this experiment showed that compared with the diabetic model group, the blood glucose in the met group decreased significantly, and there was no significant difference between the blood glucose levels in the low and medium dose LBP groups and that in the metformin group, which indicated that the hypoglycemic effect of the low and medium dose LBP was similar to that of the metformin group, which provided a basis for the later application of LBP in the clinical treatment of T2DM.

In the long-term pathological process of T2DM, in addition to IR and islet B cell dysfunction, it is also accompanied by the activation of tissue and cell immune inflammation. Inflammatory factors secreted by activated monocyte macrophages, lymphocytes and other immune cells are the pathogenic factors of inducing IR and T2DM. Compared with NC mice, the levels of IL-1 β, IL-6 and TNF - α in serum of KKAy

mice were significantly increased. IL-1 β is an important promoter of inflammatory cascade and an independent risk factor for T2DM. It is suggested that IL-1 β can activate NF - κ B and MAPKs signaling pathway to induce islet inflammatory response and apoptosis of B cells [], while the apoptosis of B cells induced by high glucose decreased when IL-1 β was knocked out [].

Inflammatory marker IL-6 is also involved in the development of T2DM. It has been reported that chronic IL-6 exposure can induce the over expression of insulin inhibitor SOCS-3, damage insulin signal transmission, and cause IR. At the same time, excessive IL-6 in the islet will cause the over activation of killer T cells, which, together with other cytotoxic effects, will cause apoptosis of islet β cells [,,]. TNF - α, as a kind of non glycosylated protein, can induce the body to produce IR by promoting the decomposition of fat granules, increasing FFA, reducing the activity of insulin receptor tyrosine kinase and interfering with normal insulin signal transduction. Therefore, this study suggests that there is a high level of inflammatory response in T2DM, and these inflammatory factors are related to the occurrence of T2DM, which is consistent with the research results of chanchira phosat et al. [] on T2DM population.

The study also found that compared with NC mice, the level of IL-10 in the serum of KKAy mice decreased. IL-10 is an anti-inflammatory factor. The decrease of serum IL-10 level is significantly related to glucose intolerance []. This is consistent with the low reactivity of IL-10 under the stimulation of high glucose in vitro experiments by Julianne C. Barry et al. TGF - β 1 plays an important role in the regulation of cell proliferation, apoptosis and immune response. At present, whether TGF - β 1 plays an anti-inflammatory or pro-inflammatory role in vivo remains controversial []. Yoshikazu naiki et al. [] found that TGF - β 1 can promote the ubiquitination and proteasome degradation of MyD88, reduce the level of MyD88 protein and delay the activation of NF - κ B by blocking the activation of TLR4 ligands, indicating that TGF - β 1 has certain anti-inflammatory effect. In this experiment, the decrease of TGF - β 1 in KKAy model mice may be related to the activation of NF - κ B signal pathway in T2DM mice, which further verified the anti-inflammatory effect of TGF - β 1.

In addition to improving the parameters of hyperglycemia, IR and other metabolic abnormalities in the body, some studies have suggested that metformin can also inhibit the secretion of pro-inflammatory molecules by macrophages by inhibiting the differentiation of monocytes into macrophages. At the same time, it can also inhibit the ratio of neutrophils and lymphocytes, the markers of inflammation to a certain extent [,,]. In this study, compared with the diabetic model group, the serum levels of IL-1 β, IL-6, TNF - α in the metformin group were significantly reduced, and the levels of anti-inflammatory factor IL-10 were significantly increased. The results were consistent with those of bobae Hyun et al. [,], indicating that metformin can improve the level of inflammation in T2DM mice and has a certain anti-inflammatory effect. After treatment with different doses of LBP, serum IL-10 increased, TNF - α decreased, and IL-1 β decreased in the low dose group. Some studies have shown that the increase of IL-10 can inhibit macrophages' secretion of proinflammatory factors, alleviate obesity mediated inflammatory response, and improve insulin sensitivity of skeletal muscle [,]. When IL-6 is deficient, it can alleviate the condition of T2DM patients to a certain extent, while for TNF - α knockout mice, IR is also relieved [,]. This shows

that LBP can inhibit the production of inflammatory factors, improve IR and alleviate T2DM, and the effect of LBP on inflammatory factors is similar to metformin. This result is consistent with the results reported by Ge JB et al. [ ] that LBP can reduce the expression of TNF - α, IL-6 and CRP in the retina of T2DM rats, play an anti-inflammatory role and alleviate the condition of T2DM rats, which provides a theoretical basis for the application of LBP in anti-inflammatory treatment of T2DM in the future.

IL-8 is an immunosuppressive factor secreted by activated monocytes. High concentration of IL-8 in serum is an important cause of late T2DM vascular complications, and also a marker of early diagnosis of diabetic nephropathy [,]. It is suggested that high glucose can stimulate the rapid rise of IL-8, which in turn can activate monocytes and neutrophils and other immune molecules, and aggravate the inflammatory damage of endothelial cells [ ]. In this study, the level of IL-8 did not change significantly. It has been suggested that in the process of TNF - α induced IR formation, IL-6 can promote insulin secretion, synergistically accelerate hyperinsulinemia, and produce toxic effects on B cells. At the same time, when there is a high level of IL-1 β in the body, it will release a large number of inflammatory factors such as TNF - α and IL-6 through the activation of inflammatory pathway to intensify the inflammatory response [ ]. However, IL-10 can inhibit NF - κ B activity by inhibiting the I κ B kinase complex, thereby reducing the expression of IL-8 [ ]. This shows that all kinds of cytokines in the body do not play a biological role alone, but there is a certain synergistic interaction, and they participate in the occurrence and development of diseases. This may also be the main reason that no obvious change of IL-8 was found in this study.

As a family member of transmembrane glycoprotein, TLR4 plays an important role in the innate immune response and the development of various diseases by mediating the release of inflammatory factors, chemokines and adhesion molecules through NF - κ B signaling pathway. TLR4 / NF - κ B is an important signaling pathway in inflammation. The activation of TLR4 / NF - κ B signaling pathway has been confirmed to be involved in the induction of adipocyte inflammation in T2DM patients. Blocking NF - κ B receptor activation signal can improve the IR state of the liver and prevent the occurrence of T2DM [].

Du Mingzhao et al. [] used 100, 250 and 500 mg / kg LBP to intervene STZ combined with high-fat diet induced diabetic SD rats, and found that compared with the normal group, NF - κ B in the kidney of the model group was highly activated, accompanied by the high expression of TNF - α, IL-6, IL-2 and other inflammatory factors in the serum. After three doses of LBP intervention, NF - κ B activation could be significantly inhibited. It is suggested that LBP can improve inflammation by inhibiting the activation of NF - κ B in T2DM rats. However, whether the inhibition is related to the inhibition of TLR4 / MyD88 dependent pathway upstream of NF - κ B has not been reported. In this study, we found that the protein levels of TLR4, MyD88, TRAF6, I κ K β, P-I κ B and nuclear NF - κ B in the model group were increased, and I κ B in the cytoplasm was decreased, suggesting that TLR4 mediated MyD88 dependent signaling pathway was activated in T2DM mice, which was consistent with the research results of Lin J and Han LP [,]. Duan D et al. [ ] mentioned that metformin can inhibit the activation of TLR4 / MyD88 / NF - κ B by activating AMPK signal pathway, and play an anti-inflammatory role. In this study, it was found that the

expression level of I κ B protein in the cytoplasm of KKAy mice increased and the expression level of other key proteins decreased after the intervention of metformin, which indicated that metformin could inhibit the TLR4 / MyD88 dependent pathway activated in T2DM mice. After the intervention of LBP, the expression of TLR4, MyD88, TRAF6, I κ K β and NF - κ B protein in the nucleus can be significantly down regulated by low and medium doses of LBP. The level of I κ B in the cytoplasm of the three dose groups is increased, and the level of P-I κ B and NF - κ B in the nucleus can be significantly inhibited by high doses of LBP, which shows that LBP, like metformin, has a certain inhibitory effect on TLR4 / MyD88 dependent signaling pathway activated in T2DM.

Conclusion

LBP has a certain hypoglycemic effect on T2DM mice, which is consistent with the human body results of others. It can regulate the inflammatory factors in T2DM mice by inhibiting TLR4 / MyD88 / NF - κ B signal pathway, so as to improve the inflammatory level of T2DM mice.

Abbreviations

LBP
Lycium barbarum polysaccharide ;
Met
Metformin ;
IL-6
Interleukin-6;
IL-1β
Interleukin − 1β;
TGF-β1
Transforming growth factor-β1;
IL-10
Interleukin − 10;
IL-8
Interleukin − 8;
TNF-α
Tumor necrosis factor − α;
TLR4
Toll-like receptors 4;
MyD88
Myeloid differentiation factor 88;
TRAF6
TNF receptor associated factor 6;
I κ Kβ

Inhibitive κ K β;
I κ B
Inhibitive κ B;
P-I κ B
Phosphorylated Inhibitive κ B;
NF - κ B
Nuclear factor-κ B;
BCA
Bicinchoninic acid ;
SDS-PAGE
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis;
PVDF
Polyvinylidene difluoride ;
T2DM
Type 2 diabetes mellitus;
MC4R
Melanocortin receptor-4 ;
GK
Glucokinase;
PK
Pyruvate kinase ;

Declarations

Ethics approval
The study protocol was reviewed and approved by the Animal Care and Use
Competing interests
The authors declare that they have no competing interests.
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Not applicable.

Authors' contributions
Design of the study was done by HY and HC. Experiments were performed by HY, SD, LB and LL. Data analyses were performed by SD, LB and TL. The manuscript was written by HY and was approved by HC. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article or are available from the corresponding author on reasonable request.

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Figures

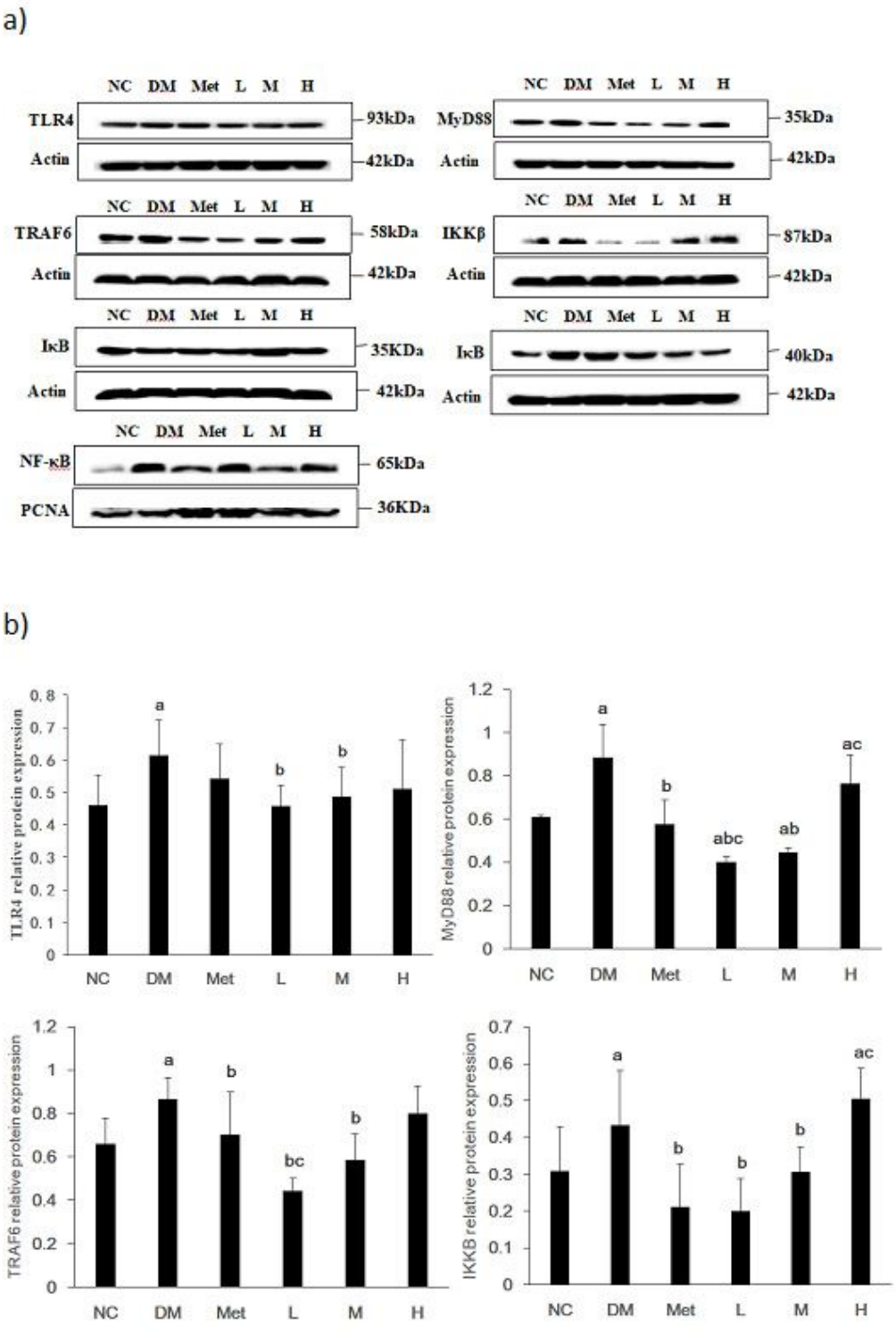

Figure 1

Fig.1. Effects of LBP on TLR4, MyD88, TRAF6, I κ K β, I κ B, P-I κ B and NF - κ B protein expression in peritoneal macrophages of T2DM mice • Data are expressed as mean ± SD. • NC, normal control; DM, diabetic model;Met, Metformin group; L, low dose LBP; M, middle dose LBP; H, high dose LBP • Pa<0.05 versus NC • Pb<0.05 versus DM • Pc<0.05 versus Met.