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)
Ningxia Med Univ, Sch Publ Hlth, Dept Nutr & Food Hyg 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

WHO global report clarifies that in 2014, prevalence of diabetes is 8.5% among adult population and 422 million people had diabetes in the world. With the prevalence of 9.4% in total adult, China has the most diabetics in the world. [1]. Many complications, such as blindness, kidney failure, low limb amputation and so on, will significantly impact on diabetic's 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 and galacturonic acid [2].

A previous study [3] demonstrated that LBP has protective effects on diabetes-associated male spermatogenic dysfunction, which was likely mediated through increased antioxidant enzyme activities and decline of cell death. [4]In addition, effect of LBP[5] was demonstrated in urine and liver metabolomics in a high-fat diet and streptozotocin-induced diabetic rat. LBP also has the function of improving cardiac hypertrophy and inhibiting expression of calpain-1 and activation of NF-κB in diabetic rats [6].

Few clinical studies investigated the protecting effect of LBP on postprandial glucose and lipid metabolism in type 2 diabetic patients [7]. And LBP reduces vascular lesions induced by T2DM through regulating p38MAPK signaling pathways and increasing antioxidative capacity [8].

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 new evidence of protection mechanism of LBP on diabetes. Therefore, in present study we attempted to explore the effects of LBP on hyperglycemia and inflammation in diabetic KKAy mice.

Materials And Methods

Preparation of LBP

As described previously [9], dry powder of *L. barbarum* was decocted with water (60 °C) by a traditional method used for Chinese medicinal herbs after degreasing. Filtered with regenerated cellulose membranes of 30 to 300kDa (0.2 MPa, 60 °C), 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 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-week 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 kept under standard conditions(22 ± 2 °C with a 12-h light/dark cycle). After feeding with high fat diet (patent in China, application number: CN201110127312.5) for 5 weeks, when the, KKAy mice with fasting blood glucose higher than 16.7 mmol L^{-1} were randomly divided into five groups (n = 10 in each group): 1) diabetic model (DM); 2) Metformin group (Met), 40mg/kg, intragastric administration; 3) low dose LBP (L), 20mg/kg, intragastric administration; 4) middle dose LBP (M), 40mg/kg, intragastric administration; and 5) high dose LBP (H), 80mg/kg, intragastric administration. The C57BL/6J mice (n = 10) were fed normal chow diet as normal control (NC). Composition of the diets is shown in table 1. NC and DM were gavaged with distilled water. Both diet and water were consumed ad libitum during 90 days' treatment. 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.

Tab 1 Composition of the diets (per 100 g)

Compositions	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 sacrificed mice were immersed in 75% ethanol solution for 2-3 minutes to disinfect the skin. RPMI1640 medium was injected into the abdominal cavity of mice in sterile environment with 1 ml syringe needle. The irrigation solution was suck out after gently squeezing the abdominal wall with hand for more than 20 times.. A small amount of cell suspension was detected by Giemsa staining light microscope. Peritoneal exudate cells were collected by centrifugation(2000r / min and 5min) and washed with Hanks for three times. RPMI1640 medium containing 15% calf serum was used to suspend the cells. The collected cells were cultured with 5% CO2 at 37 ℃ for 24 hours to make macrophages adhere to the wall. Relatively pure macrophage was obtained by washing it with PBS.

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 was 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-value < 0.05 or P-value < 0.01 were considered to be statistically significant.

Results

The weight changes of T2DM mice after LBP intervention

As can be seen in figure 1, the mean body weight of KKAy mice fed with high-fat diet was significantly higher than that of the normal mice ($P < 0.01$). During the period of treatment, the mean weight of mice in each group increased significantly ($P < 0.01$), But there was no significant difference between DM mice and other treated mice($P > 0.05$).

Changes of blood glucose in T2DM mice after LBP intervention

At the beginning of our treatment, blood glucose of KKAy has no statistical difference among the treated mice ($P > 0.05$). However, there was statistical difference in blood glucose between normal control mice and the treated mice ($P < 0.05$). After 45 days of treatment, blood glucose level in DM mice was significantly higher than that in Met and LBP treated mice ($P < 0.05$). With the LBP treatment, there was a statistical difference between DM mice and low and middle dose of LBP-treated mice ($P < 0.05$). No statistical difference existed between DM mice and high dose of LBP treated mice ($P > 0.05$) (figure 2).

Changes of serum inflammatory factors in T2DM mice after LBP intervention

In high-fat diet fed KKAy mice, levels of IL-1 β, IL-6, TNF - α in KKAy mice increased ($P < 0.05$) while as levels of IL-10 and TGF - β 1 decreased ($P < 0.05$). Serum IL-8 levels was increased in KKAy mice, but there was no statistically significant among the treated mice ($P > 0.05$). The concentrations of IL-1 β, IL-6, TNF - α and IL - 10 in Met treated mice were statistically different with that in DM mice ($P < 0.05$), indicating that metformin could effectively change the inflammatory state of T2DM mice. After treatment with LBP, the elevated expression of IL-1 β, IL-6 and TNF - α and decreased expression of TL - 10 were either abolished or significantly reduced. showing an effective change in the levels of IL-10, TNF - α and IL-1 β in LBP treated mice. The expressional change of inflammation factors in LBP treated mice exhibited similar trends with Met treated mice. .

Tab 2 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 figure 3, 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 DM mice.

After the intervention, compared with DM mice, 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 treatment, 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, high-fat diet feeding drastically increased body weight and blood glucose in KKAy mice, which exhibited the characteristics of abdominal obesity and hyperglycemia. This is consistent with the experimental results of Liu min [10]. Previous studies demonstrated that LBP reduced body weight by increasing the hypothalamic leptin level, reducing appetite and accelerating fat metabolism in SD obese rats. However, in the current study, KKAy mice showed no significant change with the treatment of LBP. 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, which 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 [11]. In KKAy mice, agouti protein encoded by ay gene will bind to melanocortin receptor MC4R to antagonize the effect of leptin [12]. Therefore, the effect of LBP on the body weight of KKAy mice might not be observed.

In this study, we demonstrated that LBP ameliorated most typical diabetic features in KKAy mice, from expression of inflammation factors to hyperglycemia. Especially the hypoglycemic activity of LBP was consistent with the research results of Zhao R. et al [13]. 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 [14]. 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 [15, 16]. The results of this experiment showed that compared with DM mice, blood glucose in Met treated mice decreased significantly. And there was no significant difference between the blood glucose levels in mice treated with low and medium dose LBP and that in Met treated mice, indicating the similar hypoglycemic activity in LBP and Met treated mice.

In the long-term pathological process of T2DM, it is also accompanied by the activation of tissue and cell immune inflammation besides IR and islet B cell dysfunction. Inflammatory factors secreted by activated monocyte macrophages. Lymphocytes and other immune cells are the pathogenic factors of inducing IR and T2DM. As compared with NC mice, the production 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 [17], while the apoptosis of B cells induced by high glucose decreased when IL-1 β was knocked out [18].

Inflammatory marker IL-6 is also involved in the development of T2DM. It has been reported that chronic IL-6 exposure may cause IR by damaging insulin signal transmission due to over expression of insulin inhibitor SOCS-3. At the same time, excessive IL-6 in islet will cause the over activation of killer T cells, which, together with other cytotoxic effects, will cause apoptosis of islet β cells [19, 20, 21]. 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 a high level of inflammatory response in KKAy mice are related to the occurrence of T2DM, which is consistent with the research results of chanchira phosat et al. [22] 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 [23]. 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 [24]. Yoshikazu naiki et al. [25] 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 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 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 [26, 27, 28]. In this study, compared with DM mice, the serum levels of IL-1 β, IL-6, TNF - α in the Met treated mice 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. [29, 30], exhibited the anti-inflammatory effect of metformin. Our results demonstrate that different doses of LBP are able to increase the level of serum IL-10 and decrease production of TNF - αand IL-1 β. Some studies have shown that increase of IL-10 is related to inhibition of proinflammatory factors in macrophages, alleviation of inflammatory response mediated by obesity , and improvement of insulin sensitivity in skeletal muscle [31, 32]. There is also evidence, showing that deficiency of IL - 6 plays an important role in alleviating the condition of T2DM patients to a certain extent. While for TNF - α knockout mice, IR is also relieved [33, 34]. In our experiments, LBP inhibited the production of inflammatory factors, improved IR and alleviated T2DM. This result is consistent with the results [35] 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, an immunosuppressive factor secreted by activated monocytes, is an important cause of late T2DM vascular complications, and also a marker of early diagnosis of diabetic nephropathy [36, 37]. Research proved that high glucose stimulated the rapid rise of IL-8, which in turn activated monocytes and neutrophils and other immune molecules, and aggravated the inflammatory damage of endothelial cells [38]. In this study, the level of IL-8 did not change significantly. It has been suggested that in the process of IR formation induced by TNF - α, IL-6 can promote insulin secretion, synergistically accelerate hyperinsulinemia, and produce toxic effects on B cells. Besides that, in the presence of high level of IL - β, our body 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 [39]. However, IL-10 can inhibit NF - κ B activity by inhibiting the I κ B kinase complex, thereby reducing the expression of IL-8 [40]. 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 [41].

Du Mingzhao et al. [42] used 100, 250 and 500mg / 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 treatment of LBP with three doses, activation of NF - κ B 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 suppression 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 DM mice 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 [43, 44]. Duan D et al. [45] 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

Similar to other studies, LBP has beneficial activity in diabetic mice. Consumption of LBP decreased blood glucose and attenuated inflammation in diabetic mice through modulation of the TLR4 / MyD88 / NF - κ B signal pathway. LBP is a potential medicine in treatment of diabetes.

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 K β: 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

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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.

Ethics approval
The study protocol was reviewed and approved by the Animal Care and Use Committee at Ningxia Med Univ (Yinchuan, China).

Consent for publication

Consent for publication –> If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent for publication.

Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details

1 Ningxia Med Univ, Sch Publ Hlth, Dept Nutr & Food Hyg, Yinchuan 750004, Ningxia, Peoples R China

2Yangzhou Univ, Sch Tourism & Cooking, Dept Nutr, Yangzhou 225000, Jiangsu, Peoples R China

3 Lianshui County Peoples Hosp

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Figures

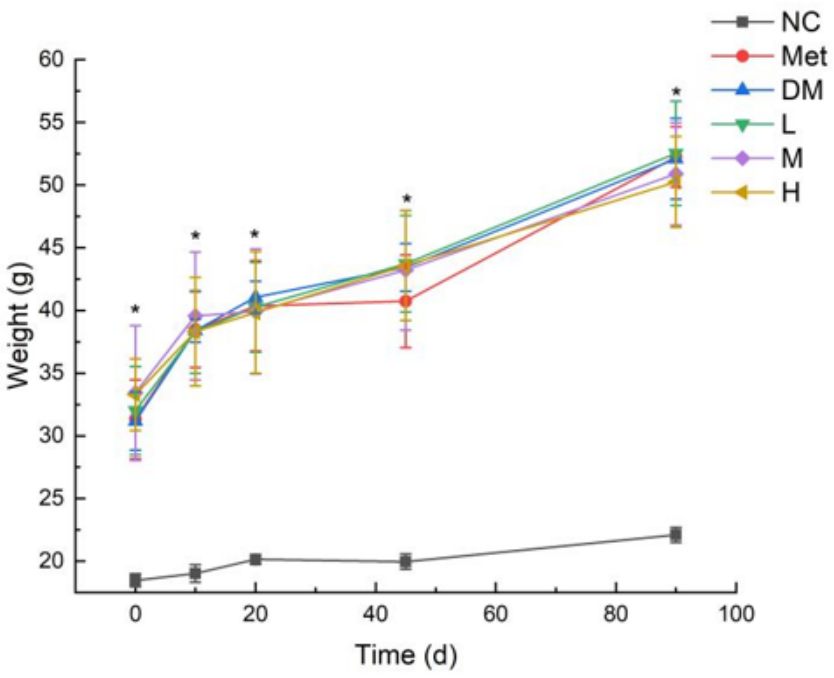

Figure 1

Effect of LBP on body weight of T2DM mice (g) • 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 • Pa<0.01 versus NC • Pb<0.05 versus DM • Pc<0.01 versus Met.

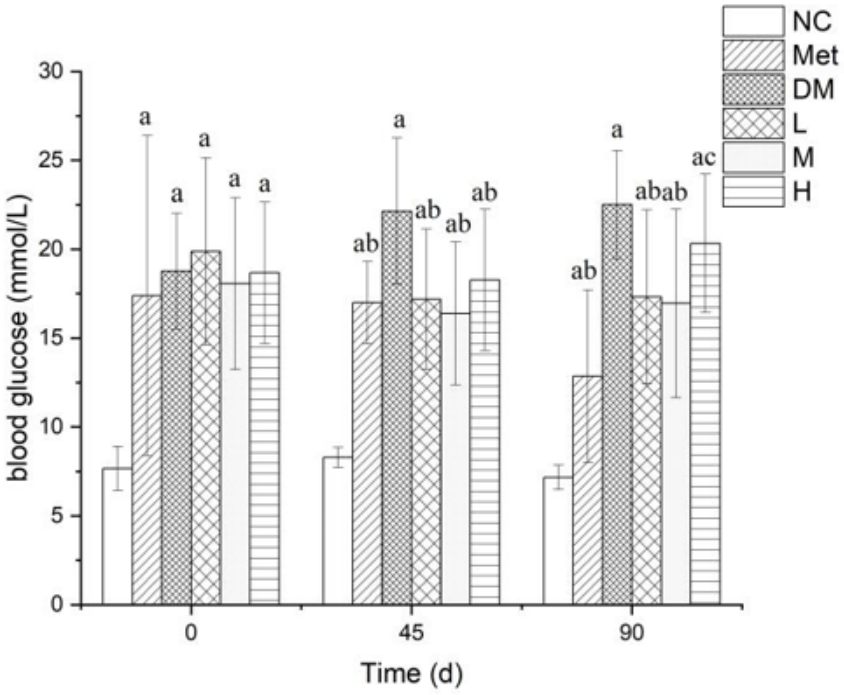

Figure 2

Effect of LBP on blood glucose in T2DM mice (mmol/L) • 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 • Pa<0.01 versus NC • Pb<0.05 versus DM • Pc<0.01 versus Met.

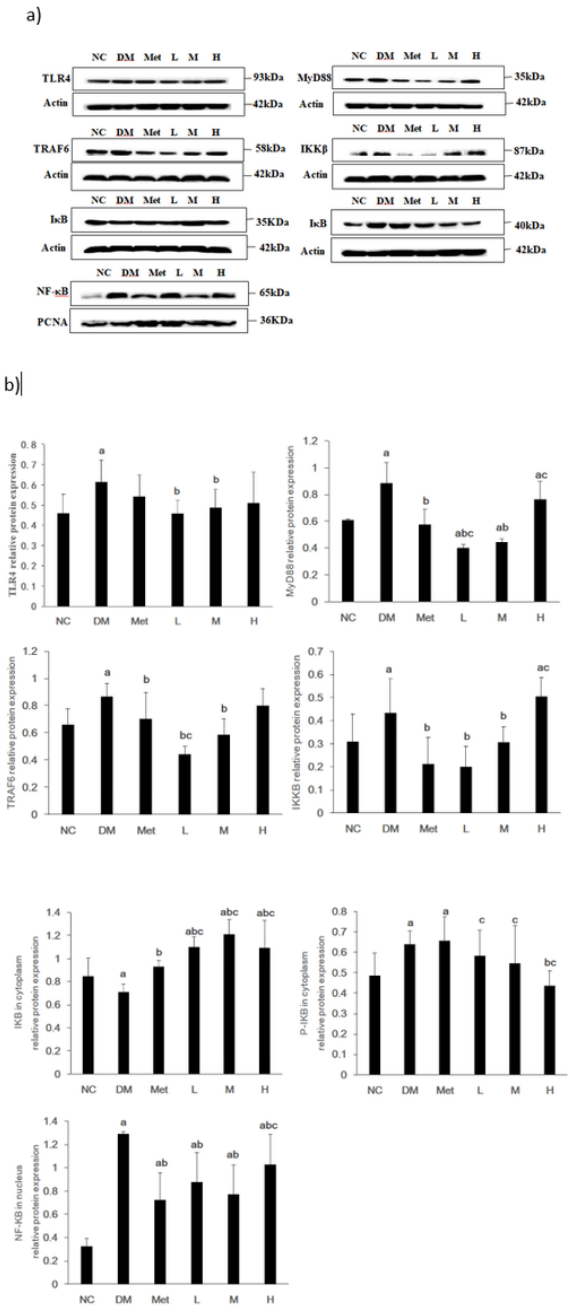

Figure 3

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.