Original Research

Polysaccharides from *Laminaria japonica* show hypoglycemic and hypolipidemic activities in mice with experimentally induced diabetes

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder of the endocrine system. The rapid increase in the incidence of DM is a serious public health concern worldwide. The treatment of DM and its complications mainly involves the use of chemically or biochemically synthesized drugs, but these drugs also have adverse side effects. Therefore, there is an urgent need to search for drugs from natural sources that would cause fewer side effects. This study aimed to determine whether polysaccharides from *Laminaria japonica* (LJP) exert hypoglycemic and hypolipidemic effects in mice with alloxan-induced diabetes. To this end, diabetes was induced by alloxan injection (200 mg/kg body weight [bw], intraperitoneal [ip]). After induction of diabetes, diabetic mice were randomly divided into five groups: diabetic control (DC) group, glibenclamide-treated (DG) group, low-dose LJP-treated (DLL) group, moderate-dose LJP-treated (DML) group, and high-dose LJP-treated (DHL) group, with normal mice used as the control group (NC group). After treatment for 28 days, body weight, fasting blood glucose (FBG), serum insulin, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels were measured. The results revealed that LJP administration prevented body-weight loss, decreased FBG levels, and increased serum insulin levels in diabetic mice. Furthermore, it decreased TC, TG, and LDL-C levels, and increased HDL-C levels in these mice. Thus, the results indicate that LJP possesses hypoglycemic and hypolipidemic activities and polysaccharides from LJP may hold promise for the development of a drug of natural origin for the treatment of DM.

Keywords: Diabetes mellitus, polysaccharides from *Laminaria japonica*, hypoglycemic, hypolipidemic, mice

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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only lead to hyperglycemia but also cause many complications, such as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis.[1–3] Thus, DM and its associated complications significantly affect health, quality of life, and life expectancy. Worldwide, the prevalence of DM in adults was estimated to be 135 million (4.0%) in 1995 and is predicted to increase to 300 million (5.4%) by the year 2025, with the prevalence being higher in developed countries than in developing countries. Furthermore, the major proportion of this increase is predicted to occur in developing countries.[4,5] Insulin and oral hypoglycemic agents, including biguanides, sulfonylureas, and thiazolidinediones, are the main treatment choices for DM, and they are effective in controlling hyperglycemia, but they also have major side effects. Therefore, there is an urgent need to search for the drugs of a natural origin with fewer side effects.[6,7]

The brown seaweed *Laminaria japonica* is the most important economic seaweed cultured in China and other countries such as Japan and Korea.[8] In East Asia, it is widely consumed as a vegetable. Furthermore, the use of *L. japonica* as a drug has been documented in traditional Chinese medicine (TCM) for over a thousand years. It has been used for its weight-reducing, detumescent, and expectorant properties in TCM.[9–11] Recent studies have shown that polysaccharides are the major biologically active components of *L. japonica*. It is well documented that *L. japonica* polysaccharides (LJP), natural macromolecular substances found in the intercellular or intracellular compartment of the seaweed, are composed of algin, laminarin, fucoidan, and different proportions of galactose, xylose, glucuronic acid, and limited protein content.[12,13] Several studies have shown that some bioactive polysaccharides isolated from marine algae

have hypoglycemic and hypolipidemic activities. For example, Yang *et al*.[14] found that polysaccharides from *Sargassum fusiforme* could decrease blood glucose, total cholesterol (TC), triglyceride (TG), and low-density lipoprotein-cholesterol (LDL-C) levels in high-fat-diet-fed rats with streptozotocin-induced diabetes. Huang *et al*.[15] reported that polysaccharides of *Spirulina platensis* and *Sargassum thunbergii* could decrease blood glucose level and could protect the vascular systems of rats with alloxan-induced diabetes. Yu *et al*.[16] found that sulfated polysaccharides of different molecular weights from *Ulva pertusa* (Chlorophyta) exhibit hypolipidemic activity. Chen *et al*.[17] reported that polysaccharides from *Ulva conglobata* Kjellm. Significantly decrease blood glucose levels in diabetic mice and restore them to normal levels, indicating an obvious dose–effect relationship. Recently, LJP has attracted considerable attention owing to its antitumor, antioxidant, antithrombotic, antiaging, antiviral, antiradiation, antifatigue, antianoxia, and hepatoprotective effects.[8,10,13,18–20] However, few studies have examined the therapeutic effects of LJP on DM. We hypothesized that LJP might have hypoglycemic and hypolipidemic effects based on the activities of other polysaccharides from marine algae. The present study was therefore designed to elucidate the hypoglycemic and hypolipidemic effects of LJP in mice with alloxan-induced diabetes.

Materials and methods

Materials

*L. japonic*a was collected from Dalian, China, during August 2010. The material was identified by Professor Li M, a botanist of Harbin Medical University (Harbin, China). A voucher specimen has been deposited in the herbarium of Harbin Medical University. The collected *L. japonic*a was washed with fresh water in order to remove salt, sand, and epiphytes, dried at 40°C, and ground into a moderately coarse powder by using a mechanical grinder.

Chemicals

Alloxan was purchased from Sigma Chemical Co. (USA). Glibenclamide was purchased from Tianjing Lisheng Pharmaceutical Co. (Tianjing, China). The kits for measuring TC (No. F002-2), TG (No. F001-2), high-density lipoprotein-cholesterol (HDL-C) (No. F003-1), and LDL-C (No. F004-2) levels were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The diagnostic kit for serum insulin (No. KMS-B05071) was purchased from Beijing Beifang Pharmaceutical Co. (Beijing, China). All other chemicals were of the highest commercial grade available on the domestic market. Freshly prepared redistilled water was used in the present study.

Preparation of LJP

LJP was prepared as previously described[21–23] with slight modifications. Briefly, *L. japonica* powder was extracted with hot water (90°C) thrice (1:40, w/v). The insoluble residue was separated from the aqueous extract by centrifugation (10,640 $\times$ g for 15 min), and the supernatant was precipitated with three volumes of 95% (v/v) ethanol at 4°C for 24 h. The precipitate was filtered and dried in an oven at 50°C for 24 h. The dried crude polysaccharides were refluxed thrice with acetone and chloroform to remove lipids. The resultant product was extracted in hot water and then filtered, and the combined filtrate was precipitated again using ethanol. The precipitate was collected and vacuum-dried to yield the desired LJP. The polysaccharide contents were determined using the anthrone–sulfuric acid reaction with glucose as a standard,[24] and the polysaccharide content of the crude extract was 26.41%. The dried LJP was dissolved in saline solution just before use.

Animals

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese National Institutes of Health. Male Kunming mice (weight, 18–22 g) were purchased from the animal laboratory of Harbin Medical University. The animals were housed with free access to standard laboratory chow and water at 23 ± 2°C with a dark and light cycle of 12 h each. The study was approved by the Institutional Ethical Committee of Harbin Medical University.

Establishing a mouse model of alloxan-induced diabetes

The mice were adapted to the test environment by feeding standard experimental diet for one week. After fasting for 24 h, the mice were injected with a freshly prepared aqueous solution of alloxan (200 mg/kg body weight [bw], intraperitoneal [ip]), as described previously.[25–27] Diabetes in mice was identified by polydipsia and polyuria, and by measuring fasting serum glucose levels 72 h after injection of alloxan. Mice having blood glucose levels higher than 11.1 mmol/L were considered diabetic and were used for the study.

Experimental design and treatment

After confirmation of the diabetic state, the mice with alloxan-induced diabetes were randomly divided into five groups (8 mice per group), and normal mice were used as the control group.

(i) Normal control (NC) group: normal mice were allowed free access to a normal diet and were treated with saline solution.
(ii) Diabetic control (DC) group: diabetic mice were allowed free access to a normal diet and treated with saline solution.
(iii) Glibenclamide-treated (DG) group: diabetic mice were put on a normal diet and treated with 4 mg/kg bw of glibenclamide.
(iv) Low-dose LJP-treated (DLL) group: diabetic mice were put on a normal diet and treated with 50 mg/kg bw of LJP.

(v) Moderate-dose LJP-treated (DML) group: diabetic mice were put on a normal diet and treated with 100 mg/kg bw of LJP.
(vi) High-dose LJP-treated (DHL) group: diabetic mice were put on a normal diet and treated with 200 mg/kg bw of LJP.

LJP and glibenclamide were dissolved in 2.0 mL of saline solution, and the control (NC and DC) groups received the same volume of saline solution. Treatments were administered orally by gavage using a feeding needle, once a day for 28 consecutive days. The rationale for the selection of the doses was based on our previous work.

Biochemical assays

The body weights and FBG levels were measured at 0 (pretrial), 7, 14, 21, and 28 days after the administration of LJP, and blood was collected from the tip of the tail vein after a 12 - to 14-h overnight fast, starting from 9:00 a.m. On the last day, the animals were not given food overnight and blood samples were collected in the test tube by quickly removing the eyeball from the socket under general anesthesia with sodium pentobarbital (40 mg/kg bw, ip) by using a pair of tissue forceps. Blood samples were allowed to clot at room temperature and then serum was immediately separated by centrifugation at 956 × g at room temperature for 10 min. Samples were stored at −70°C for the assay of serum insulin, TC, TG, LDL-C, and HDL-C levels. Upon completion of blood collection, the mice were sacrificed by cervical dislocation.

Method of measurements

Plasma glucose was measured using a Kyoto blood sugar test meter and test strip (Arkray Inc., Kyoto, Japan). The levels of TC, TG, LDL-C, and HDL-C were determined by enzyme methods.[28,29] The levels of insulin were estimated by enzyme-linked immunosorbent assay (ELISA) method.[30]

TC level was determined using cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) method; optical density was measured at 500 nm. TG level was measured using glycerol-3-phosphate oxidase *p*-aminophenol (GOP-PAP) method; optical density was measured at 546 nm. LDL-C level was determined using polyvinyl sulfate (PVS) method; optical density was measured at 500 nm. HDL-C level was measured using phospho-wolframic acid magnesium (PTA-Mg^{2+}) precipitation method; optical density was measured at 500 nm. Insulin level was measured using ELISA.

Acute toxicity test

The acute toxicity test was carried out according to the guidelines of the Organization for Economic Co-operation and Development (OECD).[31] Healthy Kunming mice (18–22 g) of either sex, fasted overnight were divided into two groups (8 mice per group) and were orally administered with LJP in increasing dose levels of 2000 and 5000 mg/kg bw. The mice were monitored for 14 days, for mortality and general behavior.

Statistical analysis

Data were expressed as mean ± standard deviation (SD), and assessed with one-way analysis of variance (ANOVA) followed by Fisher's least-significant difference (LSD) by using SPSS 15.0 software. The difference between groups was considered to be significant at $P < 0.05$.

Results

Acute toxicity studies

The various observations showed the normal behavior of the mice. No toxic effects were observed at a higher dose of 5000 g/kg bw. Hence, there were no lethal effects in any of the groups during the experimental period. Therefore, LJPs were considered practically non-toxic substances.

Effects of LJP on body weights in mice

Effects of LJP on body weights in mice are shown in Figure 1. As shown in Figure 1, before embarking on the experiments, all the groups had no significant difference in body weights ($P < 0.05$). After seven days of treatment with LJP, the body weights of the diabetic groups (DC, DG, DLL, DML, and DHL groups) were significantly lower ($P < 0.05$) than that of the NC group, 34.4%, 19.6%, 24.5%, 21.3%, and 17.3% lower, respectively. After 14 days of treatment with LJP, the body weights of the DG, DML, and DHL groups were significantly higher ($P < 0.05$) than that of the DC group. After 28 days of treatment with LJP, body weights of the DG, DLL, DML, and DHL groups were significantly higher ($P < 0.05$) than that of the DC group, 52.1%, 40.2%, 57.6%, and 63.1% higher, respectively. Thus, a clear dose-dependent increase in body weights after LJP treatment was observed.

Effects of LJP on FBG levels in mice

Effects of LJP on FBG levels in mice are shown in Figure 2. As shown in Figure 2, FBG levels of NC group were maintained constant during 28 days and were significantly lower ($P < 0.05$) than that of the diabetic groups (DC, DG, DLL, DML, and DHL groups). After 14 days of treatment with LJP, the FBG levels of the DG, DLL, DML, and DHL groups were significantly lower ($P < 0.05$) than that of the DC group. After 28 days of treatment with LJP, FBG levels of the DG, DLL, DML, and DHL groups had decreased by 117.6%, 87.2%, 141.8%, and 160.4%, respectively, when compared with that of the DC group. Thus, after LJP treatment, a clear dose-dependent decrease in FBG levels was observed.

Effects of LJP on serum insulin levels in mice

Effects of LJP on serum insulin levels in mice are shown in Figure 3. As shown in Figure 3, after 28 days of treatment with LJP, the serum insulin levels of diabetic groups (DC, DG, DLL, DML, and DHL groups) were significantly lower ($P < 0.05$) than that of the NC group. However, the serum insulin levels of the DG, DLL, DML, and DHL groups were significantly higher ($P < 0.05$) than that of the DC group, 39.5%, 26.7%, 34.9%, and 48.7% higher, respectively.

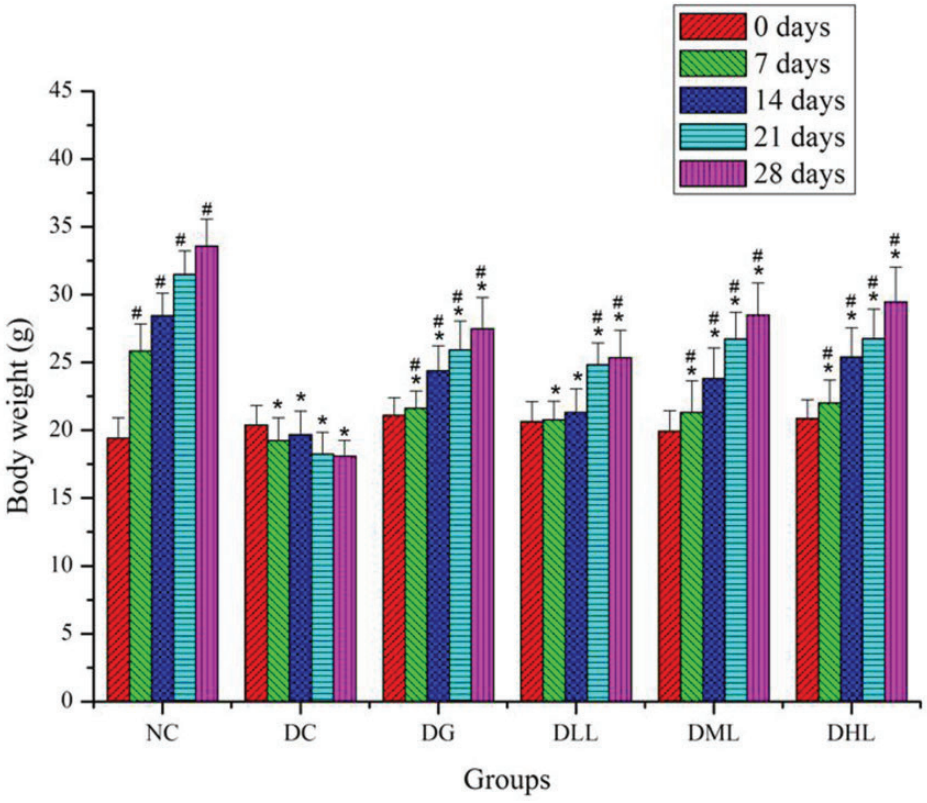

Figure 1 Effects of *Laminaria japonica* polysaccharide (LJP) on body weights in mice. Body weights were measured at 0, 7, 14, 21, and 28 days after the administration of LJP. Data are presented as mean ± SD. $^{*}P < 0.05$ as compared with normal control (NC) group. $^{\#}P < 0.05$ as compared with the diabetic control (DC) group. (A color version of this figure is available in the online journal)

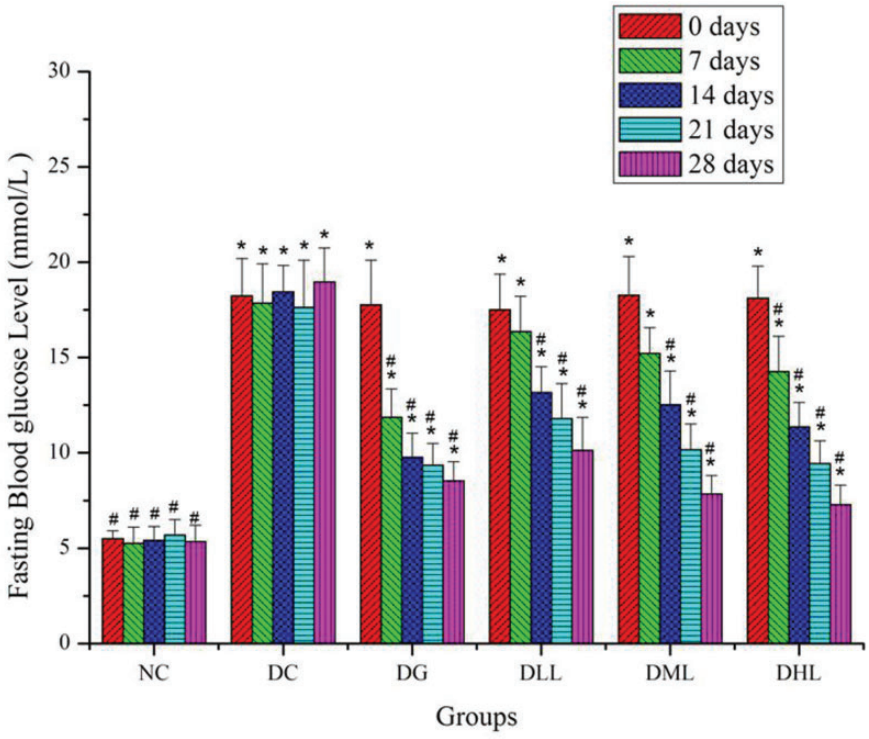

Figure 2 Effects of *Laminaria japonica* polysaccharide (LJP) on fasting blood glucose levels in mice. Fasting blood glucose levels were measured at 0, 7, 14, 21, and 28 days after the administration of LJP. Data are presented as mean ± SD. $^{*}P < 0.05$ as compared with normal control (NC) group. $^{\#}P < 0.05$ as compared with the diabetic control (DC) group. (A color version of this figure is available in the online journal)

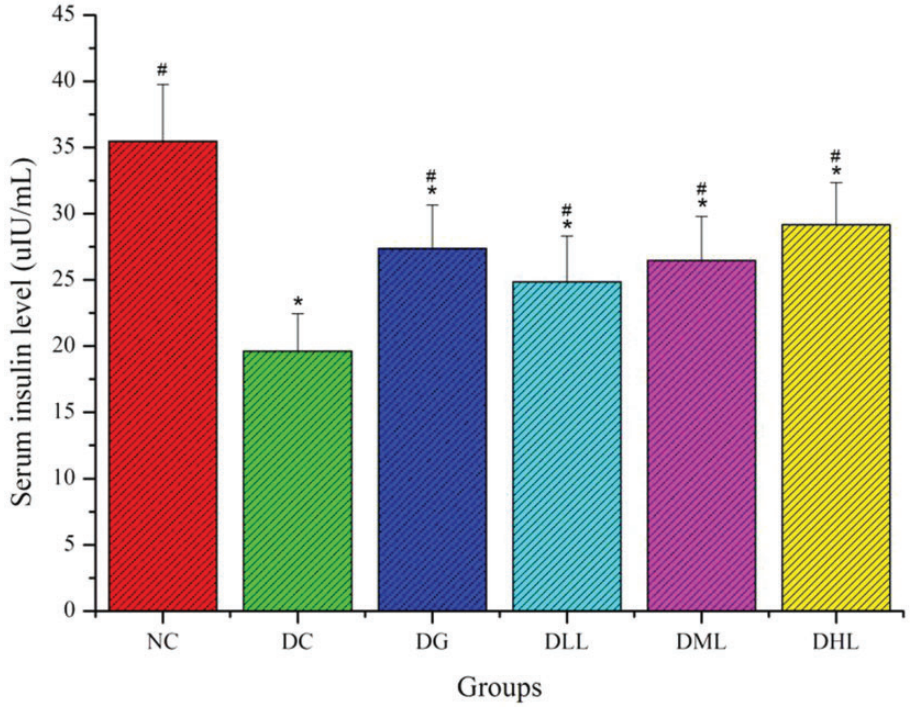

Figure 3 Effects of *Laminaria japonica* polysaccharide (LJP) on serum insulin levels in mice. Serum insulin levels were measured at 28 days after the administration of LJP. Data are presented as mean ± SD. $^{*}P < 0.05$ as compared with normal control (NC) group. $^{\#}P < 0.05$ as compared with the diabetic control (DC) group. (A color version of this figure is available in the online journal)

Thus, after LJP treatment, a clear dose-dependent increase in insulin levels was observed.

Effects of LJP on serum lipids levels in mice

Effects of LJP on serum lipids levels in mice are shown in Figure 4. As shown in Figure 4, after 28 days of treatment with LJP, the TC, TG, and LDL-C levels of diabetic groups (DC, DG, DLL, DML and DHL groups) were significantly higher ($P < 0.05$) than that of the NC group. However, the TC levels of the DG, DLL, DML, and DHL groups were significantly lower ($P < 0.05$) than that of the DC group, 140.3%, 85.9%, 107.8%, and 129.1% lower, respectively. The TG levels of the DG, DLL, DML, and DHL groups were significantly lower ($P < 0.05$) than that of the DC group, 133.3%, 75.1%, 125.6%, and 111.6% lower, respectively. LDL-C levels of the DG, DLL, DML and DHL groups were significantly lower ($P < 0.05$) than that of the DC group, 338.5%, 176.9%, 198.6%, and 269.3% lower, respectively. After 28 days of treatment with LJP, the HDL-C levels of diabetic groups (DC, DG, DLL, DML, and DHL groups) were significantly lower ($P < 0.05$) than that of the NC group. However, the HDL-C levels of the DG, DLL, DML, and DHL groups were significantly higher ($P < 0.05$) than that of the DC group, 131.7%, 80.5%, 117.1%, and 102.4% higher, respectively. Thus, a clear dose-dependent decrease in TC and LDL-C levels after LJP treatment was observed. However, the magnitude of change in TG and HDL-C levels after LJP treatment was not dose-dependent, warranting further study to elucidate the reason.

Discussion

Alloxan is a hydrophilic and chemically unstable pyrimidine derivative, which causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas.[32,33] The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to rapid destruction of the beta cells.[34–36] Alloxan-induced diabetes is characterized by severe loss in body weight and similar results were observed in the present study. This loss of body weight, possibly owing to the degeneration of adipocytes and muscle tissues to make up for the energy lost from the body because of frequent urination and excessive conversion of glycogen to glucose.[37] In the present study, diabetic groups showed significant loss of body weight and LJP treatment groups were significantly protected from this loss in body weights. The protective effect of LJP against body-weight loss may possibly be a result of its antihyperglycemic activity.

DM is a serious chronic disease. Effective control of the blood glucose level is important in preventing or reversing diabetes complications and improving the quality of life in both type 1 and type 2 DM patients.[38,39] In this study, the hypoglycemic effects of LJP in mice with alloxan-induced diabetes were established by the estimation of FBG levels as the important basal parameter for monitoring diabetes.

As previously described, alloxan could damage pancreatic beta cells, resulting in a decrease in endogenous insulin secretion, which decreased utilization of glucose by the tissues consequently.[40] The present study showed that LJP

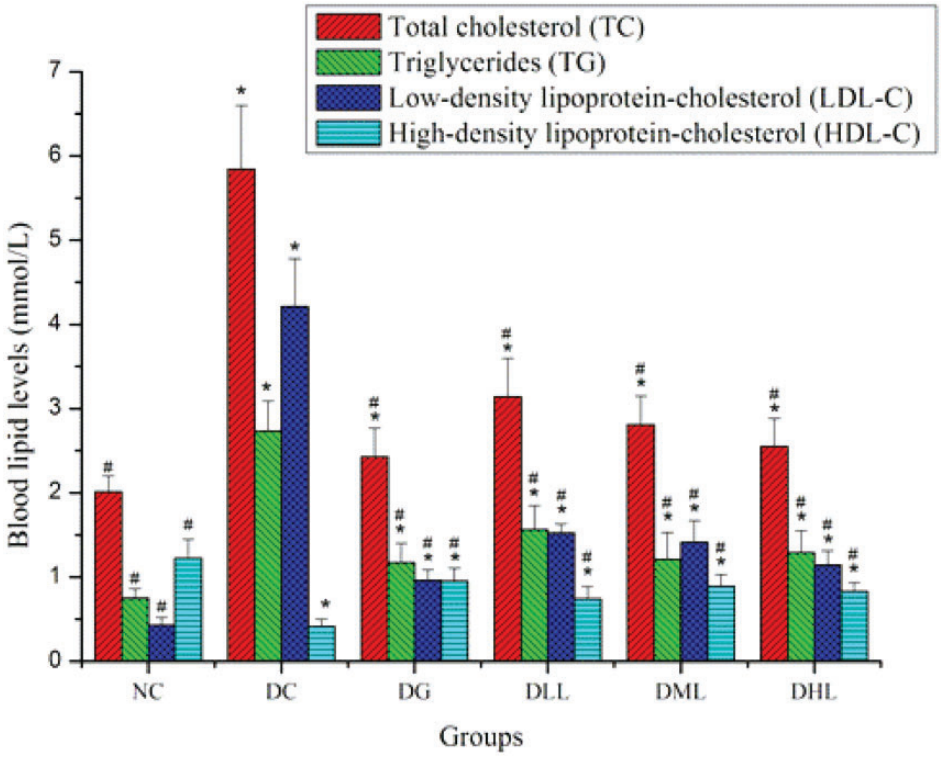

Figure 4 Effects of *Laminaria japonica* polysaccharide (LJP) on serum lipids levels in mice. Serum lipids levels were measured at 28 days after the administration of LJP. Data were presented as mean ± SD. $^{*}P < 0.05$ as compared with normal control (NC) group. $^{\#}P < 0.05$ as compared with the diabetic control (DC) group. (A color version of this figure is available in the online journal)

increased the serum insulin levels in mice with alloxan-induced diabetes. The increase in serum insulin levels may be possibly due to an effect that either increases the pancreatic secretion of insulin from the existing beta cells or an effect that releases insulin from the bound form. Additional studies are needed to confirm this hypothesis.

DM is usually associated with abnormally increased levels of serum lipids, and this increases the risk factor for cardiovascular diseases.[41] The abnormal concentration of serum lipids in diabetic subjects is mainly due to increase in the mobilization of free fatty acids from fat deposits since insulin is required for the inhibition of hormone-sensitive lipase.[42,43] The present study showed that LJP not only lowered the TC, TG, and LDL-C, but also enhanced the HDL-C, which is known to play an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed ''reverse cholesterol transport''; HDL-C is considered to be a cardio-protective lipid. The significant hypolipidemic effect of LJP may be due to an increase in insulin secretion, which, in turn, inhibits hormone-sensitive lipase and increases the utilization of glucose, and thereby decreases the mobilization of free fatty acids from fat depots.

In recent years, many investigators have endeavored to study the hypoglycemic and hypolipidemic effects of *L. japonica*. Park *et al*.[44] have discovered that *L. japonica* extract can decrease blood glucose and serum lipids in type 2 diabetic patients. Jin *et al*.[45] investigated the preventive effects of *L. japonica* extract on alterations in the activity of hepatic xanthine oxidase (XO) and oxidative stress in streptozotocin-induced diabetic rats. Long *et al*.[46] reported that *L. japonica* powder may aid in the recovery of the islet-cell secreting function and may reduce the level of FBG by an antioxidant effect. Yu *et al*.[47] found that *L. japonica* extract affects the metabolism of TG, TC, LDL, and HDL to reduce a regulation of blood lipid by enhancing the activity of lipoprotein lipase and hepatic lipase. However, the above investigators mainly studied *L. japonica* extract and powder instead of LJPs. The results of our study indicate that LJP clearly demonstrates hypoglycemic and hypolipidemic effects in mice with alloxan-induced diabetes. The hypoglycemic effect of LJP is evidenced by decreased fasting blood glucose levels and increased serum insulin levels in diabetic mice. The hypolipidemic effect of LJP is evidenced by decreased TC, TG, and LDL-C levels, and elevated HDL-C levels in diabetic mice. Although the use of LJP for diabetes treatment is promising, determining the precise molecular mechanism of the hypoglycemic and hypolipidemic effects of LJP requires further investigation. Besides, LJP still needs strict toxicological evaluation (acute and subchronic toxicity studies) to determine safety in humans.

Author contributions: All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript. XJ designed the study and wrote the paper; JY, ZW, and RX performed the research; and RL analyzed the data.

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REFERENCES

1. Luo Q, Cai Y, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. *Life Sci* 2004;**76**:137–49
2. Li FL, Li QW, Gao DW, Peng Y. The optimal extraction parameters and anti-diabetic activity of flavonoids from *Ipomoea batatas* leaf. *Afr J Tradit Complement Altern Med* 2009;**6**:195–202
3. Punitha R, Manoharan S. Antihyperglycemic and antilipidperoxidative effects of *Pongamia pinnata* (Linn.) Pierre flowers in alloxan induced diabetic rats. *J Ethnopharmacol* 2006;**105**:39–46
4. Kobayashi M, Yamazaki K, Hirao K, Oishi M, Kanatsuka A, Yamauchi M, Takagi H, Kawai K. The status of diabetes control and antidiabetic drug therapy in Japan–a cross-sectional survey of 17,000 patients with diabetes mellitus (JDDM 1). *Diabetes Res Clin Pract* 2006;**73**:198–204
5. Bazzano LA, Serdula MK, Liu S. Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr Atheroscler Rep* 2003;**5**:492–9
6. Zhang HN, He JH, Yuan L, Lin ZB. In vitro and in vivo protective effect of Ganoderma lucidum polysaccharides on alloxan-induced pancreatic islets damage. *Life Sci* 2003;**73**:2307–19
7. Yeo J, Kang YJ, Jeon SM, Jung UJ, Lee MK, Song H, Choi MS. Potential hypoglycemic effect of an ethanol extract of *Gynostemma pentaphyllum* in C57BL/KsJ-db/db mice. *J Med Food* 2008;**11**:709–16
8. Wang J, Zhang Q, Zhang Z, Song H, Li P. Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *Int J Biol Macromol* 2010;**46**:6–12
9. Li N, Zhang Q, Song J. Toxicological evaluation of fucoidan extracted from *Laminaria japonica* in Wistar rats. *Food Chem Toxicol* 2005;**43**:421–6
10. Wang J, Zhang Q, Zhang Z, Li Z. Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. *Int J Biol Macromol* 2008;**42**:127–32
11. Wang J, Zhang Q, Zhang Z, Zhang H, Niu X. Structural studies on a novel fucogalactan sulfate extracted from the brown seaweed *Laminaria japonica*. *Int J Biol Macromol* 2010;**47**:126–31
12. Zvyagintseva TN, Shevchenko NM, Nazarenko EL. Water-soluble polysaccharides of some brown algae of the Russian far-east. *J Exp Mar Biol Ecol* 2005;**320**:123–31
13. Qiong L, Jun L, Jun Y, Yinzhu Z, Xiaoyan C, Mingliang Y. The effect of *Laminaria japonica* polysaccharides on the recovery of the male rat reproductive system and mating function damaged by multiple mini-doses of ionizing radiations. *Environ Toxicol Pharmacol* 2011;**31**:286–94
14. Yang XD, Zhang J, Cui RJ. Experimental study of Sargassum fusiforme polysaccharides on insulin resistance in type two diabetes mellitus rats. *Chin J Mar Drugs* 2011;**30**:42–4
15. Huang ZX, Mei XT, Xu DH, Xu SB, Lv JY. Protective effects of polysacchride of *Spirulina platensis* and *Sargassum thunbeergii* on vascular of alloxan induced diabetic rats. *Zhongguo Zhong Yao Za Zhi* 2005;**30**:211–5
16. Yu PZ, Li N, Liu XG. Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). *Pharmacol Res* 2003;**48**:543–9
17. Chen X, Fang XB, Zhong QQ. Studies on the isolation, purification and hypoglycemic activity of an acidic polysaccharide from *Ulva conglobata kjellm*. *J Chin Inst food Sci Tech* 2008;**8**:75–9
18. Yuan ZZ, Cheng KM, Huang W, Feng DR. Study on industrialized extraction technology and function of hyperlipidemic regulating of *Laminaria japonica* polysaccharides. *Zhong Yao Cai* 2010;**33**:1795–8
19. Kim KH, Kim YW, Kim HB, Lee BJ, Lee DS. Anti-apoptotic activity of laminarin polysaccharides and their enzymatically hydrolyzed oligosaccharides from *Laminaria japonica*. *Biotechnol Lett* 2006;**28**:439–46
20. Xie L, Chen MH, Li J, Yang XM, Huang QJ. Antithrombotic effect of a polysaccharide fraction from *Laminaria japonica* from the South China Sea. *Phytother Res* 2011;**25**:1362–6
21. Luo Q, Liu J, Yan J, Cui XY. *Laminaria japonica* polysaccharides on the recovery of rats' spermatogenic function of testis damaged by chronic local ionizing radiation. *J Med Plants Res* 2010;**4**:1400–6
22. Gao MX, Ye S. Research on the extraction of polysaccharide in the Kelp. *J Yangtze Univ* 2005;**2**:73–5
23. Li LY, Li LQ, Guo CH. Evaluation of in vitro antioxidant and antibacterial activities of *Laminaria japonica* polysaccharide. *J Med Plants Res* 2010;**4**:2194–8
24. Morris DL. Quantitative determination of carbohydrates with dreywood's anthrone reagent. *Science* 1948;**107**:254–5
25. Li F, Tang H, Xiao F, Gong J, Peng Y, Meng X. Protective effect of salidroside from *Rhodiolae Radix* on diabetes-induced oxidative stress in mice. *Molecules* 2011;**16**:9912–24
26. Chen H, Feng R, Guo Y, Sun L, Jiang J. Hypoglycemic effects of aqueous extract of Rhizoma Polygonati Odorati in mice and rats. *J Ethnopharmacol* 2001;**74**:225–9
27. Shu XS, Lv JH, Tao J, Li GM, Li HD, Ma N. Antihyperglycemic effects of total flavonoids from Polygonatum odoratum in STZ and alloxan-induced diabetic rats. *J Ethnopharmacol* 2009;**124**:539–43
28. Ren XY, Li YN, Qi JS, Niu T. Peroxynitrite-induced protein nitration contributes to liver mitochondrial damage in diabetic rats. *J Diabetes Complications* 2008;**22**:357–64
29. Zhang QH, Wu CF, Duan L, Yang JY. Protective effects of total saponins from stem and leaf of *Panax ginseng* against cyclophosphamide-induced genotoxicity and apoptosis in mouse bone marrow cells and peripheral lymphocyte cells. *Food Chem Toxicol* 2008;**46**:293–302
30. Gong F, Li F, Zhang L, Li J, Zhang Z, Wang G. Hypoglycemic effects of crude polysaccharide from Purslane. *Int J Mol Sci* 2009;**10**:880–8
31. Sikarwar MS, Patil MB. Antidiabetic activity of *Crateva nurvala stem bark* extracts in alloxan-induced diabetic rats. *J Pharm Bioallied Sci* 2010;**2**:18–21
32. Kumarappan CT, Mandal SC. Polyphenolic extract of Ichnocarpus frutescens attenuates diabetic complications in streptozotocin-treated diabetic rat. *Ren Fail* 2008;**30**:307–22
33. Lenzen S, Panten U. Alloxan: history and mechanism of action. *Diabetologia* 1988;**31**:337–42
34. Bhagwat DA, Killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbent*. *Int J Green Pharm* 2008;**2**:126–8
35. Saravanan G, Pari L. Effect of Cogent db, a herbal drug, on serum and tissue lipid metabolism in experimental hyperglycaemic rats. *Diabetes Obes Metab* 2003;**3**:156–62
36. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia* 2008;**51**:216–26
37. Ene AC, Nwankwo EA, Samdi LM. Alloxan-induced diabetes in rats and the effects of black caraway (*Carum carvi L.*) oil on their body weight. *J Pharmacol Toxicol* 2008;**3**:141–6
38. Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan CS. Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. *Diabetes* 2002;**51**:1851–8
39. Xie JT, Wang A, Mehendale S, Wu J, Aung HH, Dey L, Qiu S, Yuan CS. Anti-diabetic effects of *Gymnema yunnanense* extract. *Pharmacol Res* 2003;**47**:323–9
40. El-Missiry MA, Othman AI, Amer MA. L-Arginine ameliorates oxidative stress in alloxan-induced experimental diabetes mellitus. *J Appl Toxicol* 2004;**24**:93–7
41. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J Biosci* 2003;**28**:1–5
42. Sri Balasubashini M, Rukkumani R, Menon VP. Protective effects of ferulic acid on hyperlipidemic diabetic rats. *Acta Diabetol* 2003;**40**:118–22
43. Ramesh B, Pugalendi KV. Antihyperlipidemic and antidiabetic effects of umbelliferone in streptozotocin diabetic rats. *Yale J Biol Med* 2005;**78**:189–96

44. Park MJ, Ryu HK, Han JS. Effects of *Laminaria japonica* extract supplement on blood glucose, serum lipids and antioxidant systems in type II diabetic patients. *J Korean Soc Food Sci Nutr* 2007;**36**:1391–8
45. Jin DQ, Li G, Kim JS, Yong CS, Kim JA, Huh K. Preventive effects of *Laminaria japonica* aqueous extract on the oxidative stress and xanthine oxidase activity in streptozotocin-induced diabetic rat liver. *Biol Pharm Bull* 2004;**27**:1037–40
46. Long SH, Yu ZQ, Shuai L, Guo YL, Duan DL, Xu XY, Li XD. The hypoglycemic effect of the kelp on diabetes mellitus model induced by alloxan in rats. *Int J Mol Sci* 2012;**13**:3354–65
47. Yu ZQ, Liu ZB, Gong SL, Dong LZ. Effects of *Laminaria japonica* on serum lipid in rats with experimental hyperlipemia. *Acta Acad Med Qingdao Univ* 2010;**46**:419–21

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