Original Article

Effects of Kudzu Root on Oxidative Stress and Inflammation in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Background: Oxidative stress and inflammation are strictly connected, and both perform an important role in the pathogenesis of diabetes mellitus (DM).

Objectives: This research aimed to investigate the potential protective effect of kudzu root against oxidative stress and inflammation in a streptozotocin (STZ)-induced DM animal model.

Methods: DM was induced in male Wistar rats by intraperitoneal injection of STZ (50 mg/kg body weight). The kudzu root (100 mg/kg BW) was administered orally after 1 week of STZ administration in diabetic animals (for 6 weeks).

Results: The diabetic animals exhibited a significant increase in fasting blood glucose, tumor necrosis factor-alpha, and malondialdehyde levels. However, they exhibited a significant decrease in plasma insulin level, superoxide dismutase, and glutathione peroxidase activity. Administration of kudzu root to diabetic animals reversed these effects.

Conclusion: The current study indicated that kudzu root has potent antidiabetic properties, likely through its anti-inflammatory and anti-oxidative properties in the STZ-diabetic rat model.

Keywords: Antioxidant, Diabetes mellitus, Inflammation, Kudzu root, Oxidative stress

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1. Introduction

Diabetes mellitus (DM) is a worldwide health issue and one of the most prevalent metabolic disorders (Kharroubi & Darwish, 2015; Moghtadaei Khorasgani et al., 2021). The hallmark of DM is chronic hyperglycemia due to a deficiency in insulin secretion and or effect (Kharroubi & Darwish, 2015). Because of its serious vascular, neurological, and infectious complications, DM is one of the most important reasons for patients' morbidity and mortality (Kharroubi & Darwish, 2015). More than 340 million people live with diabetes worldwide, predicted to increase to 578 million by 2030 (Saeedi et al., 2019).

Oxidative stress is triggered by an imbalance between free radical generation, mainly reactive oxygen species (ROS) and nitrogen reactive species (RNS), and the level of antioxidant substances, so the antioxidant system cannot scavenge the pro-oxidant species in the body (Hussain & Tan, 2016). Although a low level of ROS serves as a key signaling mediator in the regulation of several physiologic pathways in living cells, its overproduction could contribute to the oxidation of cellular macromolecules and also promotes cell and tissue damage (Rastogi & Haldar, 2018).

Oxidative stress causes several inflammatory conditions. Oxidative stress and inflammation are strictly connected, and both perform an important function in the pathogenesis of several chronic diseases (Biswas, 2016, Kaywanloo et al., 2022), such as DM and its various complications (Hussain & Tan, 2016).

Numerous pharmaceutical effects of natural products have drawn much attention for designing drugs to treat several diseases (Gao et al., 2016; Panche et al., 2016). Evidence has demonstrated that flavonoids, a subclass of polyphenols, have various valuable properties on human health mostly because of their potent antioxidant and anti-inflammatory properties (Panche et al., 2016; Karak, 2019). Kudzu root or *Pueraria lobata* has been commonly used in traditional medicine for the treatment of some disorders, in particular metabolic diseases. It has been confirmed that isoflavonoids are one of the main bioactive components in kudzu root (Gao et al., 2016). The most abundant isoflavonoid in the root of *P. lobata* is puerarin (Panche et al., 2016). The beneficial properties of puerarin have been mentioned in various metabolic disorders (Zhou et al., 2014).

Streptozotocin (STZ) suppresses insulin secretion and triggers DM (Aloud et al., 2017). In our research, diabetes was induced by STZ injection in male Wistar rats. Regarding the association between oxidative stress, inflammation, and DM, our study was designed to examine the potential effect of flavonoid-enriched- kudzu root against oxidative stress and inflammation in STZ-diabetic animals. We intended to provide evidence for using kudzu root as an effective medication for diabetes mellitus.

2. Materials and Methods

Study materials

The kudzu root powder and STZ were purchased from Sigma Co., Ltd. (Missouri, USA). Insulin enzyme-linked immunosorbent assay (ELISA) kit was obtained from MyBioSource Co. (USA). The plasma glutathione peroxidase (GPx), tumor necrosis factor-alpha (TNF-α), and malondialdehyde (MDA) ELISA kits were purchased from Hangzhou Eastbiopharm Co. Ltd. (Zhejiang, China). Superoxide dismutase (SOD) ELISA kit was provided by Bioassay Technology Laboratory Co. (Shanghai, China).

Experimental animals

A total of 21 male Wistar rats (weight: 220-240 g) were obtained from the Animal Center of Pasteur Institute of Iran/North Research Center, Iran. The animals were housed in an air-conditioned environment under standard temperatures (22˚C±2˚C) and a 12-h light–dark period. Rats were fed a standard rat chow and allowed free access to purified water.

Diabetic animal model and pharmacological intervention

The animals were randomly allocated into 3 groups, each containing 7 rats: normal control group (NC), diabetic control (DC) group, and diabetic+kudzu root (D/KR) (100 mg/kg) group. The STZ (55 mg kg) was dissolved in citrate buffer (0.05 M, pH=4.5) and intraperitoneally injected into rats. The diabetic animals were intragastrically treated with kudzu root powder 1 week after STZ injection (for 6 weeks). The NC and DC groups were treated with an equivalent volume of normal saline. Blood samples were collected by tail vein puncture 72 h after STZ administration, fasting blood glucose (FBG) concentration was investigated, and animals with FBG levels of ≥16.7 mmol/L were selected as appropriate diabetic models and used in this research. When the study was completed, the animals were fasted for 6 h, anesthetized using ketamine-xylazine, and the blood samples were collected by cardiac puncture to perform biochemical analysis.

FBG measurement

FBG level was measured using a Glucocard 01 blood glucose monitor (Arcary, USA, Inc).

Insulin measurement

According to the manufacturer's instructions, plasma insulin levels were determined in duplicate by commercial enzyme-linked immunosorbent assay (ELISA) kits from Monobind Inc. Concurrently with these tests, standards at varying concentrations were run. The insulin levels were measured according to the standard curve.

Measurement of antioxidant and oxidative stress markers

According to kit instructions, GPx activity and MDA concentration were measured in plasma samples. Plasma SOD was assayed in duplicate using a commercial ELISA kit following the manufacturer's recommendations.

Measurement of TNF-α

Plasma TNF-α level was measured in duplicate by a commercial ELISA kit (EastBiopharma) with a 1.52 ng/L sensitivity.

Statistical analysis

The data were analyzed using SPSS software, Version 20. The quantitative parameters were reported as Mean±SEM. The independent t-test was used to compare the parameters between groups. A $P<0.05$ was taken as statistically significant.

3. Results

Kudzu root improved FBG levels and body weight

At the end of the study, diabetic animals showed a higher level of FBG. Moreover, the rats that received the kudzu root represented decreased FBG levels ($P<0.05$) as compared to the diabetic rats (Figure 1).

Effect of kudzu root on plasma levels of insulin

As expected, STZ administration intensely decreased the plasma concentration of insulin. In contrast, kudzu root administration reverses the reduction of insulin in diabetic rats (Figure 2).

Kudzu root decreased MDA and increased SOD and GPx activity

Kudzu root administration could reverse the effects of diabetic condition on the MDA and SOD concentration and GPx activity so that kudzu root treatment significantly decreased MDA level and increased SOD and GPx activity in the diabetic rats ($P<0.05$) (Figure 3).

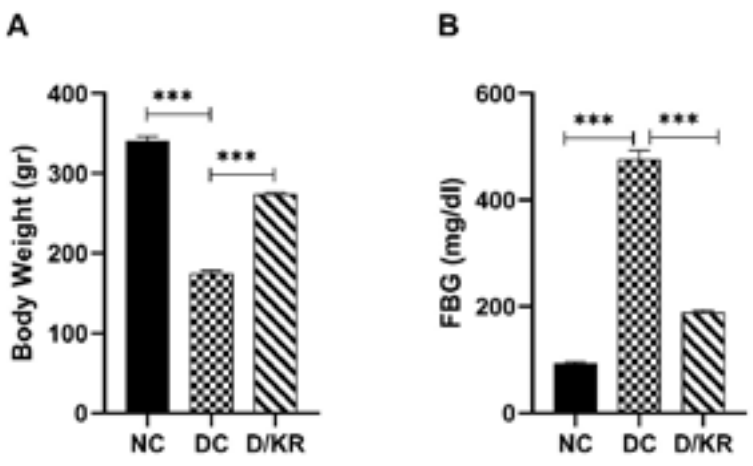

Figure 1. The effect of kudzu root on FBG levels and body weight

A) total body weight, B) FBG

Abbreviations: NC: Normal control group; DC: Diabetic control group; D/KR: Diabetic+kudzu root (D/KR) (100 mg/kg).

Data are represented as Mean±Sem. ***P<0.001.

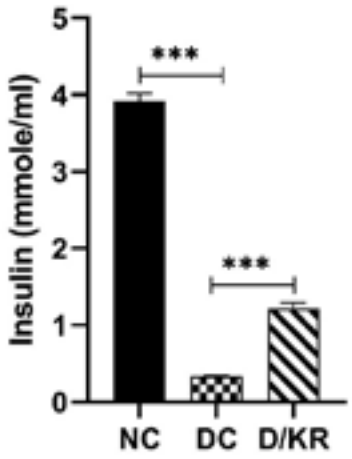

Figure 2. The effect of kudzu root on insulin levels

Abbreviations: NC: Normal control group; DC: Diabetic control group; D/KR: Diabetic+kudzu root (D/KR) (100 mg/kg).

Data are represented as Mean±Sem. ***P<0.001.

Kudzu root decreased TNF-α level

The present research demonstrated a significant enhancement (P<0.05) of plasma TNF-α in diabetic animals. Kudzu root treatment showed obvious reducing effects on plasma TNF-α levels in diabetic rats (P<0.05) (Figure 4).

4. Discussion

DM is a common metabolic condition associated with chronic inflammation and oxidative stress. Studies have revealed that STZ treatment can cause permanent DM in animal models (Wu & Yan, 2015). This research showed that insulin level was significantly decreased, and the blood sugar level noticeably increased in diabetic rats. In contrast, administration of kudzu root led to a significant decrease in plasma glucose and a significant rise in plasma insulin in STZ-diabetic rats. In this regard, several studies have reported that flavonoids can lead to glucose level reduction through glucose absorption inhibition and glucose tolerance improvement by stimulating insulin secretion and function (Fang et al., 2008, Jung et al., 2004).

Kudzu root is a rich source of isoflavones, mainly containing puerarin, daidzein, and genistein (Duru et al., 2020). A recent study revealed that isoflavone-rich kudzu extracts improved glucose and HbA1c levels and stimulated β-cells regeneration (Duru et al., 2020).

Increased oxidative stress and inflammation are key mechanisms in the pathogenesis of numerous chronic disorders, including DM (Marseglia et al., 2015). It has been shown that ROS generation is related to macromolecules and cellular damage in the pathogenesis of diabetes mellitus. Uncontrolled lipid peroxidation due to enhanced endogenous oxidant species impairs cell mem-

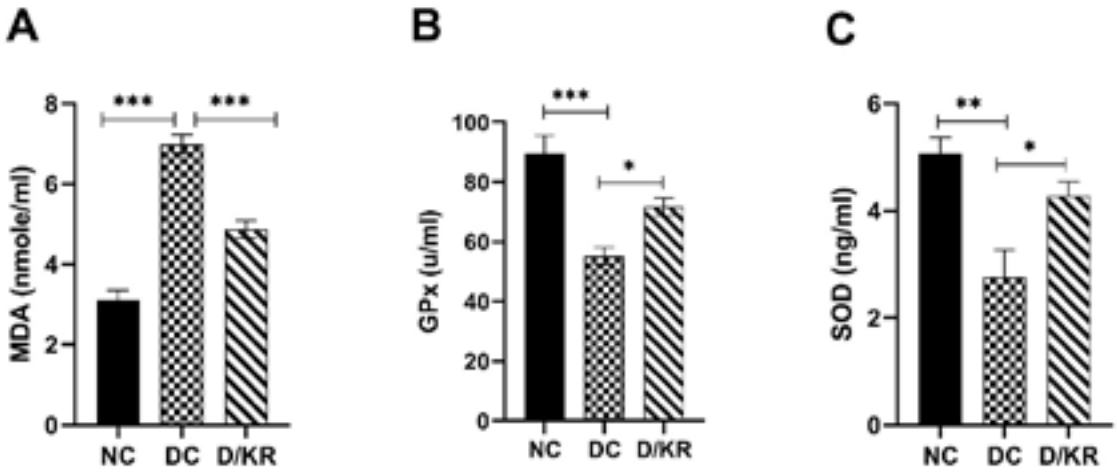

Figure 3. The effect of kudzu root on antioxidant and oxidative stress markers

A) The effects of STZ and kudzu root on MDA level activity, B) The effects of STZ and kudzu root on GPx activity, C) The effects of STZ and kudzu root on SOD activity

Abbreviations: NC: Normal control group; DC: Diabetic control group; D/KR: Diabetic+kudzu root (D/KR) (100 mg/kg).

Data are represented as Mean±SEM. ***P<0.001, **P<0.01, *P<0.05.

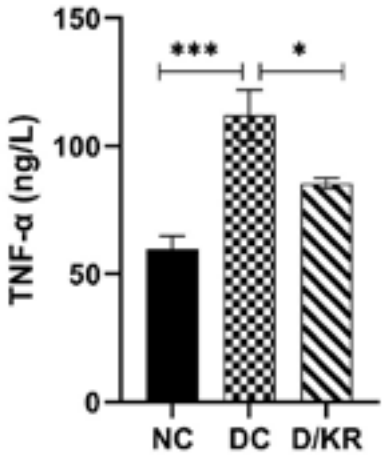

Figure 4. The effect of kudzu root on TNF-α

Abbreviations: NC: Normal control group; DC: Diabetic control group; D/KR: Diabetic+kudzu root (D/KR) (100 mg/kg).

Data are represented as Mean±SEM. ***P<0.001, *P<0.05.

brane functioning. Altogether, lipid peroxidation can lead to cellular infiltration, islet cell damage, and the development of DM (Asmat et al., 2016). As an important product of lipid peroxidation, MDA considerably rises, as described in the previous studies on STZ-induced diabetic rat models (Sheweita et al., 2016). In the current study, the MDA levels revealed a significant increase in STZ-induced diabetic animals, and the administration of kudzu root significantly reversed this effect.

However, the body has antioxidant defenses against the effect of oxidants species (Birben et al., 2012). SOD is a key anti-oxidative enzyme that acts as the first line of defense against ROS to reduce lipid peroxidation and oxidative stress by catalyzing the conversion of superoxide radicals into H_2O_2 that is detoxified by the activities of glutathione peroxidase (Gpx) and catalase (CAT). In addition, enzymatic and non-enzymatic antioxidants are important in inhibiting cellular oxidative stress. Glutathione (GSH) is the most common non-enzymatic endogenous antioxidant, which serves as a substrate for GPx and is a direct scavenger of ROS/RNS. The previous research has shown decreased plasma levels of GSH and SOD in STZ-induced diabetic animals as compared to the control group, which is likely due to reduced synthesis or augmented degradation of GSH by oxidative stress (Sheweita et al., 2016, Xie et al., 2018). In the present study, GPx activity was significantly reduced in diabetic animals.

Although some studies have suggested that kudzu root has potent antioxidant properties in some disorders, the evidence supporting the antioxidant effects of kudzu root in DM conditions is limited. In this regard, it has been revealed that puerarin, one constituent of kudzu root, considerably reverses the reduction in SOD activity and rise in MDA levels in arthritis animal models (Wang et al., 2016). Another study shows that kudzu root significantly protects the rat pheochromocytoma cell line (PC12) cells against H_2O_2 damage by increasing the CAT and SOD activities and glutathione levels (Zhang et al., 2017). A study also demonstrates that puerarin, isolated from kudzu root, can increase manganese SOD and CAT activities in a diabetic nephropathy model in STZ-diabetic mice (Xu et al., 2016). In this study, kudzu root administration decreased MDA levels and increased the activity of antioxidant enzymes, SOD, and GPx in STZ-induced diabetic rats.

Studies have revealed that chronic inflammation has a key function in the pathogenesis of metabolic disease, so pro-inflammatory cytokines are identified to be raised in several metabolic disorders such as DM (Tsalamandris et al., 2019). Among pro-inflammatory cytokines, TNF-α is one of the major cytokines that initiates inflammatory processes. Therefore, there has been a growing interest in targeting inflammation as a means to prevent and treat metabolic disorders (Tsalamandris et al., 2019). In this regard, the increased level of TNF-α cytokine in the plasma of diabetic rats may contribute to β cell and insulin dysfunction. The level of this cytokine significantly decreased in diabetic rats after kudzu root administration. This result is in accordance with the previous reports that several flavonoids have been shown to inhibit pro-inflammatory cytokine production in diabetic conditions (Samie et al., 2018; Ginwala et al., 2019). The anti-inflammatory of kudzu root was also shown in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells (Jin et al., 2012). It has also been revealed that genistein and puerarin effectively ameliorate alcohol-induced hepatic injury through antioxidant and anti-inflammatory actions in mice (Zhao et al., 2016). A recent study showed that puerarin can reduce the expression of TNF-α and improve insulin resistance in the gestational DM rat model (Xu et al., 2020).

5. Conclusion

In conclusion, while further evidence is required to precisely determine exactly how kudzu root affects DM, this research revealed that the antidiabetic action of kudzu root is likely attributed to its anti-inflammatory and anti-oxidative properties in the STZ-diabetic rat model.

Ethical Considerations

Compliance with ethical guidelines

The Research and the Medical Ethics Committee of Shahroud University of Medical Sciences approved the experimental protocol in this study (Code: IR.SHMU.REC.1398.022).

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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مقاله پژوهشی

تأثیر ریشه کودزو بر استرس اکسیداتیو و التهاب در رت‌های دیابتی القاشده با استرپتوزوتوسین

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چکیده

زمینه مطالعه: استرس اکسیداتیو و التهاب به‌شدت با هم مرتبط هستند. هر دوی آن‌ها نقش مهمی در پاتوژنز دیابت شیرین دارند.

هدف: در این مطالعه اثر محافظتی بالقوه ریشه کودزو در برابر استرس اکسیداتیو و التهاب در مدل حیوانی دیابت ملیتوس القاشده با استرپتوزوتوسین بررسی شده است.

روش کار: دیابت ملیتوس در موش‌های صحرایی نر نژاد ویستار با تزریق داخل صفاقی استرپتوزوتوسین (۵۰ میلی‌گرم بر کیلوگرم وزن بدن) ایجاد شد. ریشه کودزو (۱۰۰ میلی‌گرم بر کیلوگرم وزن بدن) پس از گذشت ۱ هفته از تجویز استرپتوزوتوسین، در حیوانات دیابتی (به‌مدت ۶ هفته) به‌صورت خوراکی تجویز شد.

نتایج: حیوانات دیابتی افزایش معناداری در سطوح گلوکز خون ناشتا، فاکتور نکروز تومور آلفا و مالون دی‌آلدئید نشان دادند، اما کاهش معناداری در سطح انسولین پلاسما و سوپراکسید دیسموتاز و فعالیت گلوتاتیون پراکسیداز داشتند. تجویز ریشه کودز به حیوانات دیابتی توانست این اثرات را معکوس کند.

نتیجه‌گیری نهایی: ریشه کودزو دارای خاصیت ضددیابتی احتمالاً از طریق خواص ضدالتهابی و ضداکسیداتیو در مدل حیوانی دیابت القاشده با استرپتوزوتوسین است.

کلیدواژه‌ها: آنتی‌اکسیدان، دیابت، التهاب، ریشه کودزو، استرس اکسیداتیو

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