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

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ABSTRACT

BACKGROUND: It has been demonstrated that oxidative stress and inflammation are strictly connected and both of them perform an important role in the pathogenesis of diabetes mellitus (DM)

OBJECTIVES: The purpose of this research was to investigate the potential protective effect of kudzu root against oxidative stress and inflammation in streptozotocin (STZ)-induced DM animal model

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

RESULTS: The diabetic animals exhibited significant increase in fasting blood glucose (FBG), tumor necrosis factor alpha (TNF-α), malondialdehyde (MDA) levels, however, they exhibited significant decrease in plasma insulin level and superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. Administration of kudzu root to diabetic animals could reverse these effects. **CONCLUSIONS:** The current study indicated that kudzu root has potent antidiabetic properties likely through its anti-inflammatory and anti-oxidative properties in STZ-diabetic rat model.

KEY WORDS: Antioxidant, Diabetes Mellitus, Inflammation, Kudzu Root, Oxidative Stress

Introduction

Diabetes mellitus (DM), is a one of the most prevalent metabolic disorders and a major health issue worldwide (Kharroubi and Darwish, 2015, Moghtadaei Khorasgani *et al*., 2021). The hallmark of DM is chronic hyperglycemia due to a deficiency in insulin secretion and/or action (Kharroubi and Darwish, 2015). Because of its serious complications including vascular, neurological, and infectious, DM is documented as one of the most important reasons for morbidity and mortality (Kharroubi and Darwish, 2015). More than 340 million people are living with diabetes worldwide, and it has been predicted to increase to 578 million people by 2030 (Saeedi *et al*., 2019).

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

Oxidative stress significantly causes several inflammatory conditions. It has been demonstrated that oxidative stress and inflammation are strictly connected and both of them perform an important function in the pathogenesis of several chronic diseases (Biswas, 2016, Kaywanloo *et al* 2022) such as DM and various complications of this disorder (Hussain and Tan, 2016).

Because of numerous pharmaceutical effects of natural products have attracted a great deal of 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, promote a wide range of 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 disorders. 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 Pueraria lobata is puerarin (Panche *et al*., 2016). The beneficial properties of puerarin have been indicated on the 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 designed to examine the potential effect of flavonoid-enriched-kudzu root against oxidative stress and inflammation in STZ-diabetic animals to provide evidence for using kudzu root as an effective medication for diabetes mellitus.

Materials and methods

Materials

Sigma Co., Ltd. (Missouri, USA) provided the kudzu root powder and STZ. 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. Superoxide dismutase (SOD) ELISA kit was provided by Bioassay Technology Laboratory CO. (Shanghai, China).

Experimental animals

A total of 21 male Wistar rats with 220– 240 g of weight 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 ± 2°C) and a 12-hour light–dark period. Rats were fed a standard rat chow and allowed free access to purified water. The experimental protocol in this study was approved by Research and the Medical Ethics Committees of Shahroud University of Medical Sciences (IR.SHMU.REC.1398.022).

Diabetic animal model and pharmacological intervention

The animals were randomly allocated into three groups each containing seven rats: normal control group (NC), diabetic control (DC) group and diabetic+kudzu root (D/KR) (100 mg/kg) group. The STZ (55 mg kg) dissolved in a 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). Accordingly, the NC and DC groups were treated with equivalent volume of normal saline. Blood samples were collected by tail vein puncture 72h after STZ administration, fasting blood glucose (FBG) concentration was investigated and animals with FBG level of ≥16.7 mmol/L were selected as appropriate diabetic models and used in this research. When the study period is completed, the animals were fasted for 6 hours, anesthetized using ketamine-xylazine, and the blood samples were collected by cardiac puncture in order to perform biochemical analysis. The experimental protocol in this study was approved by Research and the Medical Ethics Committees of Shahroud University of Medical Sciences.

FBG measurement

FBG level was measured using an 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. Parallel to the tests, standards at varying concentrations were run. The insulin levels were measured according to the standard curve.

Measurement of antioxidant and oxidative stress markers

GPx activity and MDA concentration were measured in plasma samples according to kit instructions. 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 commercial ELISA kit (EastBiopharma) with sensitivity 1.52 ng/L.

Statistical analysis

The data were analyzed using SPSS 20 software. The quantitative parameters were reported as mean±SEM. Independent t-test was used to compare the parameters between groups. A p-value <0.05 was taken as statistically significant.

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 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 plasma concentration of insulin. In contrast, kudzu root administration reverse reduction of insulin in diabetic rats. (Figure **2**).

Kudzu root decreased MDA and increased SOD and GPx activity

Kudzu root administration was able to reverse the effects of diabetic condition on the MDA and SOD concentration and GPx activity so that kudzu root treatment significantly led to reduction of MDA level, and increased SOD and GPx activity in diabetic rat ($P<0.05$) (Figure **3**).

Kudzu root decreased TNF-α level

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

Discussion

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

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

Increased oxidative stress and inflammation accepted to play a central role 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 damages in the pathogenesis of diabetes mellitus. Uncontrolled lipid peroxidation due to enhanced endogenous oxidant species impairs cell membrane functioning. Altogether, lipid peroxidation can lead to cellular infiltration, islet cell damage and development of DM (Asmat *et al*., 2016). As an important product of lipid peroxidation, MDA has revealed an important rise 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 administration of kudzu root significantly reversed this effect.

To counteract oxidant species, the body has antioxidant defenses against the effect of oxidants (Birben *et al*., 2012). SOD is a key anti-oxidative enzyme which acts as first line of defense against ROS to reduce lipid peroxidation and oxidative stress (17) by catalyzing the conversion of superoxide radicals into H2O2 that is detoxified by the activities of glutathione peroxidase (Gpx) and catalase (CAT). In addition, the enzymatic and non-enzymatic antioxidants play an important function in inhibiting cellular oxidative

stress. The 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 (18). The previous researches have shown decreased plasma levels of GSH and SOD in STZ induced diabetic animals as compared to 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 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 condition is limited. In this regard, it has been revealed that puerarin, one constituent of kudzu root, considerably reverse reduction in SOD activity and rise in MDA level in arthritis animal model (Wang *et al*., 2016). Another study showed that kudzu root significantly protects the rat pheochromocytoma cell line (PC12) cells against H_2O_2 damage through increasing the CAT and SOD activities and glutathione levels (Zhang *et al*., 2017). A study also demonstrated that puerarin, isolated from kudzu root, can increase manganese SOD (MnSOD) and CAT activities in a diabetic nephropathy model in STZ-diabetic mice (Xu *et al*., 2016). In this study, kudzu root administration led to decrease in MDA level and increase in 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 that 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 initiate inflammatory processes. Therefore, it has produced growing interest in targeting inflammation in order to prevent and treat metabolic disorder (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 was significantly decreased in the diabetic rats after kudzu root administration. This result is in accordance with the previous reports that a number of 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 gestational diabetes mellitus (GDM) rat model (Xu *et al*., 2020).

Conclusion

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

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تاثیر ریشه کودزو بر استرس اکسیداتیو و التهاب در رت های دیابتی القا شده با استرپتوزوتوسین

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چکیده

زمینه مطالعه: استرس اکسیداتیو و التهاب به شدت با هم مرتبط هستند و هر دوی آنها نقش مهمی در پاتوژنز دیابت شیرین (DM)دارند.

هدف: بررسی اثر محافظتی بالقوه ریشه کودزو در برابر استرس اکسیداتیو و التهاب در مدل حیوانی دیابت ملیتوس (DM) القا شده با استرپتوزوتوسین (STZ)

روش کار: دیابت ملیتوس، در موش های صحرایی نر نژاد ویستار با تزریق داخل صفاقی STZ (50 میلی گرم بر کیلوگرم وزن بدن) ایجاد شد. ریشه کودزو (100 میلی گرم بر کیلوگرم وزن بدن) پس از گذشت 1 هفته از تجویزSTZ ، در حیوانات دیابتی (به مدت 6 هفته) به صورت خوراکی تجویز شد.

نتایج: حیوانات دیابتی افزایش معنی‌داری در سطوح گلوکز خون ناشتا (FBG)، فاکتور نکروز تومور آلفا (TNF-α)، و مالون دی‌آلدئید (MDA) نشان دادند، اما کاهش معنی‌داری در سطح انسولین پلاسما و سوپراکسید دیسموتاز (SOD) و فعالیت گلوتاتیون پراکسیداز (GPx) داشتند. تجویز ریشه کودز به حیوانات دیابتی توانست این اثرات را معکوس کند.

نتیجه‌گیری نهایی: ریشه کودزو دارای خاصیت ضد دیابتی احتمالاً از طریق خواص ضد التهابی و ضد اکسیداتیو در مدل حیوانی دیابت القا شده با STZ است.

Figures

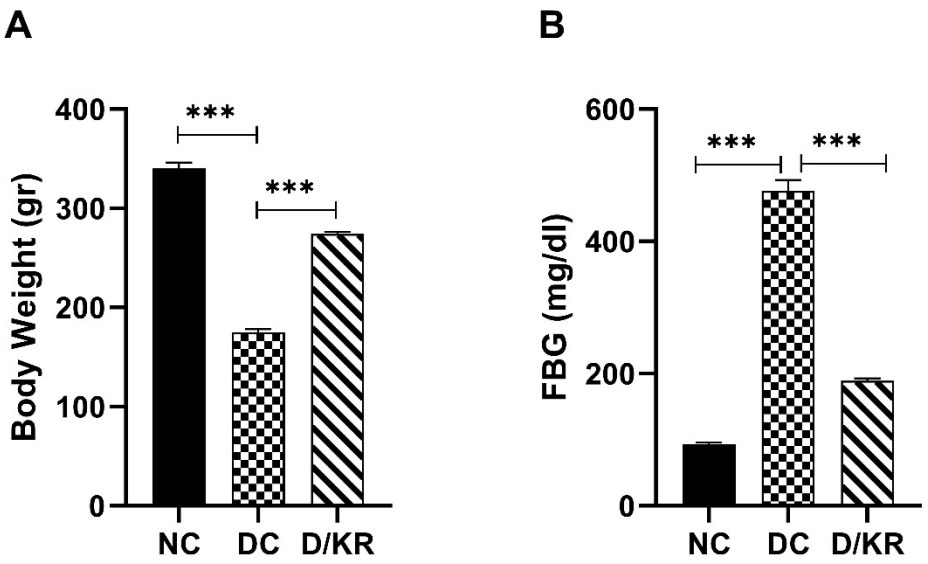

Figure 1: The effect of kudzu root on FBG levels and body weight. The animals were randomly allocated into three groups consisting of seven animals each: normal control group (NC), diabetic control (DC) group and diabetic+kudzu root (D/KR) (100 mg/kg). The effects of STZ and Kudzu root on total body weight (A) and fasting blood glucose (FBG) (B) are represented.Data are represented as mean ± SEM. ***: $p<0.001$, **: $p<0.01$, *: $p<0.05$.

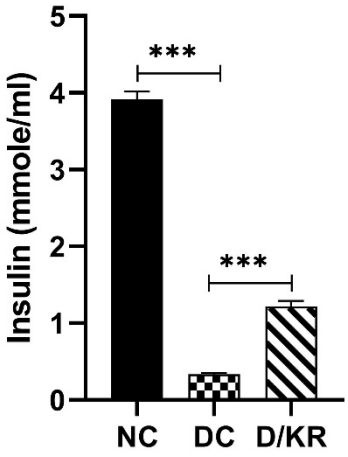

Figure 2: The effect of kudzu root on insulin levels. The animals were randomly allocated into three groups consisting of seven animals each: normal control group (NC), diabetic control (DC) group and diabetic+kudzu root (D/KR) (100 mg/kg). The effects of STZ and Kudzu root on insulin level is represented. Data are represented as mean ± SEM. ***: p<0.001, **: p<0.01, *: p<0.05.

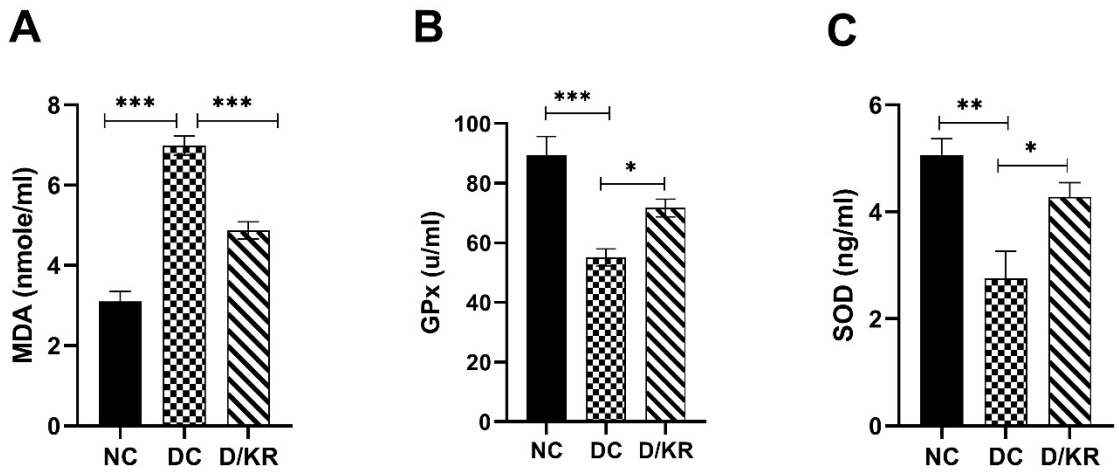

Figure 3: The effect of kudzu root on antioxidant and oxidative stress markers. The animals were randomly allocated into three groups consisting of seven animals each: normal control group (NC), diabetic control (DC) group and diabetic+kudzu root (D/KR) (100 mg/kg). The effects of STZ and Kudzu root on malondialdehyde (MDA) level (A), glutathione peroxidase (GPx) (B) and superoxide dismutase (SOD) (C) activity are represented. Data are represented as mean ± SEM. ***: p<0.001, **: p<0.01, *: p<0.05.

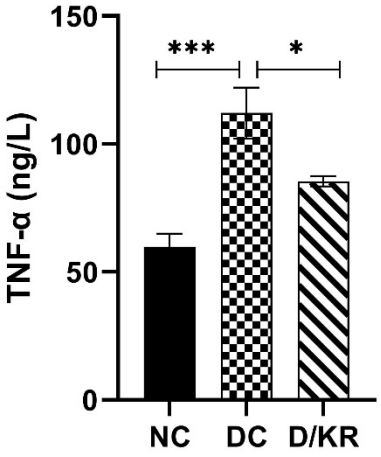

Figure 4: The effect of kudzu root on TNF-α. The animals were randomly allocated into three groups consisting of seven animals each: normal control group (NC), diabetic control (DC) group and diabetic+kudzu root (D/KR) (100 mg/kg). The effects of STZ and Kudzu root on tumor necrosis factor alpha (TNF-α) level is represented.Data are represented as mean ± SEM. ***: p<0.001, **: p<0.01, *: p<0.05.

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