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เทตระไฮโดรเคอร์คูมิน ลดระดับน้ำตาลในเลือดสูงในหนูที่เป็นเบาหวาน ผ่านการลดความต้านทานต่ออินซูลินและความเครียดออกซิเดชัน

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Tetrahydrocurcumin Attenuates Hyperglycemia in Diabetic Rats Through Reduction of Insulin Resistance and Oxidative Stress

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หลักการและวัตถุประสงค์: โรคเบาหวานเป็นปัญหาสาธารณสุขที่สำคัญระดับโลก โดยมีสาเหตุเกิดจากความผิดปกติของการหลั่งและการออกฤทธิ์ของอินซูลิน โรคเบาหวานก่อให้เกิดภาวะแทรกซ้อนอันเป็นสาเหตุนำไปสู่การเสียชีวิตในผู้ป่วยเบาหวาน การศึกษานี้มีวัตถุประสงค์เพื่อประเมินผลของสารเทตระไฮโดรเคอร์คูมิน (tetrahydrocurcumin, THU) ซึ่งเป็นสารต้านอนุมูลอิสระที่มีฤทธิ์รุนแรง ต่อระดับน้ำตาลในเลือด ระดับอินซูลิน ความต้านทานต่ออินซูลิน และความเครียดออกซิเดชันในหนูที่เป็นเบาหวานด้วย streptozotocin-nicotinamide (STZ-NA)

วิธีการ: หนูสายพันธุ์วิสตาร์เพศผู้ จำนวนสี่สิบตัวได้รับการสุ่มแบ่งออกเป็น 5 กลุ่ม ได้แก่ 1) กลุ่มควบคุม 2) กลุ่มควบคุมที่รักษาด้วย THU (100 มก./กก./วัน) 3) กลุ่มเบาหวาน 4) กลุ่มเบาหวานที่รักษาด้วย THU (50 มก./กก./วัน) และ 5) กลุ่มเบาหวานที่รักษาด้วย THU (100 มก. /กก. /วัน) หนูถูกเหนี่ยวให้เกิดโรคเบาหวานด้วยการฉีด STZ ขนาด 55 มก./กก./วัน หลังจากได้รับ NA ขนาด 110 มก. /กก./วัน หลังจากนั้น THU จะถูกป้อนเวลาแปดสัปดาห์ เมื่อครบกำหนด ระดับกลูโคสในพลาสมา ระดับอินซูลิน ความไวต่อการตอบสนองของอินซูลิน และตัวชี้วัดความเครียดออกซิเดชันได้แก่มาลอนไดอัลดีไฮด์และโปรตีนคาร์บอนิลในพลาสมา รวมถึงสารต้านอนุมูลอิสระกลูตาไธโอนได้รับการประเมิน

ผลการศึกษา: หนูที่เป็นโรคเบาหวานมีภาวะน้ำตาลในเลือดสูงร่วมกับภาวะความต้านทานต่ออินซูลินและพบความบกพร่อง

Background and Objective: Diabetes is a major global public health problem caused by impaired insulin secretion and action. The complications of diabetes are the leading cause of death in diabetic patients. In the present study, the effects of tetrahydrocurcumin (THU), a strong antioxidant are assessed on plasma glucose, insulin levels, insulin resistance, and oxidative stress in streptozotocin-nicotinamide (STZ-NA) diabetic rats.

Methods: Forty male Wistar rats were randomly divided into five groups: 1) control, 2) control treated with THU (100 mg/kg/day), 3) diabetic, 4) diabetic treated with THU 50 mg/kg/day, and 5) diabetic treated with THU 100 mg/kg/day. Diabetes was induced by injection of STZ 55 mg/kg/day after a dose of NA 110 mg/kg/day. Eight weeks after oral administration of THU, plasma glucose levels, insulin levels, insulin sensitivity, oxidative stress markers including plasma malondialdehyde (MDA) and plasma protein carbonyl, and antioxidant glutathione (GSH) levels were evaluated.

Results: Diabetic rats showed hyperglycemia, insulin resistance, and impaired glucose tolerance, ($p<0.05$ compared with non-diabetic rats). These alterations

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ในการควบคุมน้ำตาลกลูโคส (p<0.05 เมื่อเทียบกับหนูที่ไม่เป็นเบาหวาน) การเปลี่ยนแปลงเหล่านี้พบร่วมกับการเพิ่มขึ้นของภาวะความเครียดออกซิเดชันเนื่องจากพบหลักฐานการเพิ่มขึ้นของมาลอนไดอัลดีไฮด์และโปรตีนคาร์บอนิลในพลาสมา (p<0.05) นอกจากนี้ยังพบการลดลงอย่างมีนัยสำคัญของสารต้านอนุมูลอิสระกลูตาไธโอนภายในเซลล์ของหนูเบาหวาน การรักษาด้วย THU พบว่าสามารถลดระดับระดับน้ำตาลในเลือดขณะอดอาหาร ช่วยเพิ่มความไวต่อการตอบสนองของอินซูลินและบรรเทาความเครียดออกซิเดชันในหนูที่เป็นเบาหวาน

สรุป: THU สามารถลดระดับน้ำตาลในเลือดในหนูที่มีภาวะเบาหวาน โดยกลไกของ THU อาจเกี่ยวข้องกับฤทธิ์ลดระดับน้ำตาลโดยการเพิ่มความไวต่อการตอบสนองของอินซูลินและฤทธิ์ต้านอนุมูลอิสระของ THU

คำสำคัญ: เทตระไฮโดรเคอร์คูมิน; น้ำตาลในเลือดสูง; หนูที่เป็นเบาหวาน; ความต้านทานต่ออินซูลิน; ความเครียดออกซิเดชัน

were associated with oxidative stress, as the evidence of increasing MDA and protein carbonyl (p<0.05). Moreover, intracellular GSH were significantly decreased in those rats. Treatment with THU in dose-dependently decreased fasting blood glucose levels, improved insulin sensitivity, and alleviated oxidative stress in diabetic rats.

Conclusions: THU exerts a beneficial effect on minimizing hyperglycemia in diabetic rats. The mechanism might involve the glucose-lowering effect by improving insulin sensitivity, and antioxidant activity of THU.

Keywords: Tetrahydrocurcumin; Hyperglycemia; Diabetic Rats; Insulin Resistance; Oxidative Stress

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Introduction

Diabetes mellitus (DM) is a non-communicable disease and considered to be a major global public health problem. The pathophysiology of DM is associated with the disorder of insulin homeostasis[1]. DM is mainly categorized as type 1 DM and type 2 DM. Type 1 DM is an absolute deficiency of insulin production. The latter is much more prevalent, type 2 DM, is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. Chronic hyperglycemia is a result of both types. It plays an important role in the pathogenesis of diabetic vascular complications such as retinopathy, nephropathy, neuropathy, cardiomyopathy, and vasculopathy[2, 3]. Poor treatment or prevention of these complications is the leading cause of death in diabetic patients. Oxidative stress is the major underlying mechanism of hyperglycemia-accelerated diabetic complications. Several studies revealed that under diabetic conditions, excessive generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-) is enhanced. O_2^- directly quenches various functional biomolecules especially cellular lipid and protein leading to cellular damage and dysfunction. In addition, oxidative stress potentially disrupts insulin secretion from pancreatic β-cells and induces insulin resistance[4, 5]. Therefore, attenuation of hyperglycemia, insulin resistance, and oxidative stress in diabetic patients is one of the strategies to prevent the development or progression of diabetic complications.

Tetrahydrocurcumin (THU), a major bioactive metabolite of curcumin (*Curcuma Longa*), has been reported to have a variety of pharmacological properties especially, antioxidant, anti-inflammatory, and organ-protective properties[6-8]. The structure of THU includes a phenol and a β-diketone functional group, which is a common structural feature found on antioxidative molecules[8-10]. The previous studies demonstrated that administration of THU for 8 weeks mitigates oxidative stress and prevents end-organ damages in toxic metal-overloaded animals[8].

In addition, treatment with THU in animal models of diabetes shows a significant reduction in insulin resistance, plasma free fatty acids, and alleviation of altered carbohydrate metabolic enzymes[9].

Hence, there is not enough evidence about the efficiency of the antioxidative effect and antidiabetic property of THU. In the present study, we investigated the beneficial effect of THU in the animal model of type 2 diabetes. The type 2 diabetic rats were induced by administration of streptozotocin (STZ) and nicotinamide (NA)[11, 12]. STZ exerts cytotoxic actions on the pancreatic β-cells, whereas NA prevents the diabetogenic effect of STZ. Rats treated with STZ and NA manifest symptoms of type 2 diabetes. We hypothesized that administration of THU for 8 weeks (treatment duration follows the previous study[8]) could alleviate hyperglycemia, insulin resistance, and oxidative stress in these animals.

Materials and Methods

Drugs and chemicals

THU (≥ 95.0% purity by HPLC) were generously provided by The Government Pharmaceutical Organization, Bangkok, Thailand. Streptozotocin and nicotinamide were purchased from Sigma-Aldrich, St. Louis, Missouri, USA.

Animals

Eight-week-old male Wistar rats weighing 180-200 grams (n=40) were obtained from the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. All animals were housed in the HVAC (Heating, Ventilation, and Air-Conditioning) System with 12 hours'dark/light cycle at the Northeast Laboratory Animal Center Khon Kaen University, Thailand.

After acclimatization, rats were randomly divided into five groups, each group n=8: control, control treated with THU (100 mg/kg/day), diabetic, diabetic treated with THU 50 mg/kg/day (low dose), and diabetic treated with THU 100 mg/kg/day (high dose). THU at dose 50 or 100 mg/kg/day was orally administered to rats for 8 weeks.

The experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 96/62).

Induction of animal model of type 2 diabetes

Diabetes was induced in overnight fasted rats. Rats were peritoneally injected with NA (110 mg/kg/day) followed by peritoneal injection of STZ (55 mg/kg/day). Only rats with fasting plasma glucose higher than 200 mg/dL at 48 hr. after STZ-NA injection were used in the following experiments.

Metabolic parameters measurements

Blood glucose levels were determined by a glucometer with a strip (Accu-Chek, Roche). The plasma insulin level was evaluated by using ELISA assay kits (Millipore Corporation, Billerica, MA, USA). A homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows.

HOMA-IR= *fasting blood glucose (mg/dL) × insulin (μIU/mL)/405*

Fasting blood glucose levels and intraperitoneal glucose tolerance test

Following the overnight fasted for at least 8 hr., fasting blood glucose was detected by a glucometer with a strip (Accu-Chek, Roche). After that, a glucose load at dose 2 g/kg body weight (25% glucose solution) was intraperitoneally injected into the rats, and blood glucose levels were measured at 15, 30, 60, and 120 minutes after glucose loading for determining glucose tolerance.

Assessment of oxidative stress markers

The levels of plasma malondialdehyde (MDA) and protein carbonyl were measured following previously described methods[8].

Assay of glutathione (GSH) in whole blood was performed by previously described methods[13] with some modifications[8, 10].

Statistical analysis

Results were expressed as mean ± SEM of measurement. The differences among various groups were compared by using one-way analysis of variance (ANOVA) followed by a post-hoc Turkey test. A value of $p< 0.05$ was considered statistically significant.

Results

Effect of THU on metabolic parameters in diabetic rats.

Injection of STZ-NA significantly retarded the increase in weight gain of diabetic rats compared with normal controls ($p<0.05$; 71.79±7.66 vs. 91.63± 3.63%; Table 1). Treatment with THU for eight weeks significantly increased the body weight gain in those rats ($p<0.05$; 71.79±7.66 vs. 86.96 ± 8.38% (diabetes+THU100; Table 1).

It appeared that at the end of the experiment, fasting blood glucose and HOMA-IR of diabetic rats were increased while serum insulin levels had no significant difference between the diabetic groups (Table 1). These results suggested that the injection of STZ-NA successfully mimics the clinical manifestation of type 2 diabetes.

Treatment with THU, especially at a high dose, was able to attenuate an elevated fasting blood glucose and alleviate insulin resistance by reducing HOMA-IR (Table 1).

Effect of THU on intraperitoneal glucose tolerance test in diabetic rats.

The intraperitoneal glucose tolerance test (IPGTT) was performed to evaluate the ability of diabetic rats to metabolize glucose after the administration of THU

for 8 weeks. The results revealed that before induction of diabetes, there were no significant differences in IPGTT among the group tested (Fig. 1A).

After 8 weeks of diabetic induction; diabetic, diabetic treated with THU 50 mg/kg/day and 100 mg/kg/day/day rats showed a significantly higher basal glucose level than normal controls (289.93 ± 1.30, 264.12 ± 1.41, and 224.62 ± 1.39 mg/dL vs. 93.74 ± 1.96 mg/dL respectively, Table 1). The glucose concentration in most of the groups increased at 30 min and decreased slowly. At 60-120 min after glucose administration, the serum glucose levels of normal controls and diabetic treated with THU 100 mg/kg/day were normalized to approximately their basal level in except the diabetic rats and diabetic treated THU 50 mg/kg/day (Fig. 1B).

The AUC analysis revealed significant differences between control and diabetic rats ($p<0.05$) (Fig. 1D). THU at 100 mg/kg/day supplementation significantly reduced the AUC value of diabetic rats ($p<0.05$, Fig. 1D).

Effect of THU on oxidative stress and antioxidant in diabetic rats.

Oxidative stress is well-recognized as one of the major players in the pathogenesis of insulin resistance and diabetic vascular complications.

A marked increase in the plasma malondialdehyde

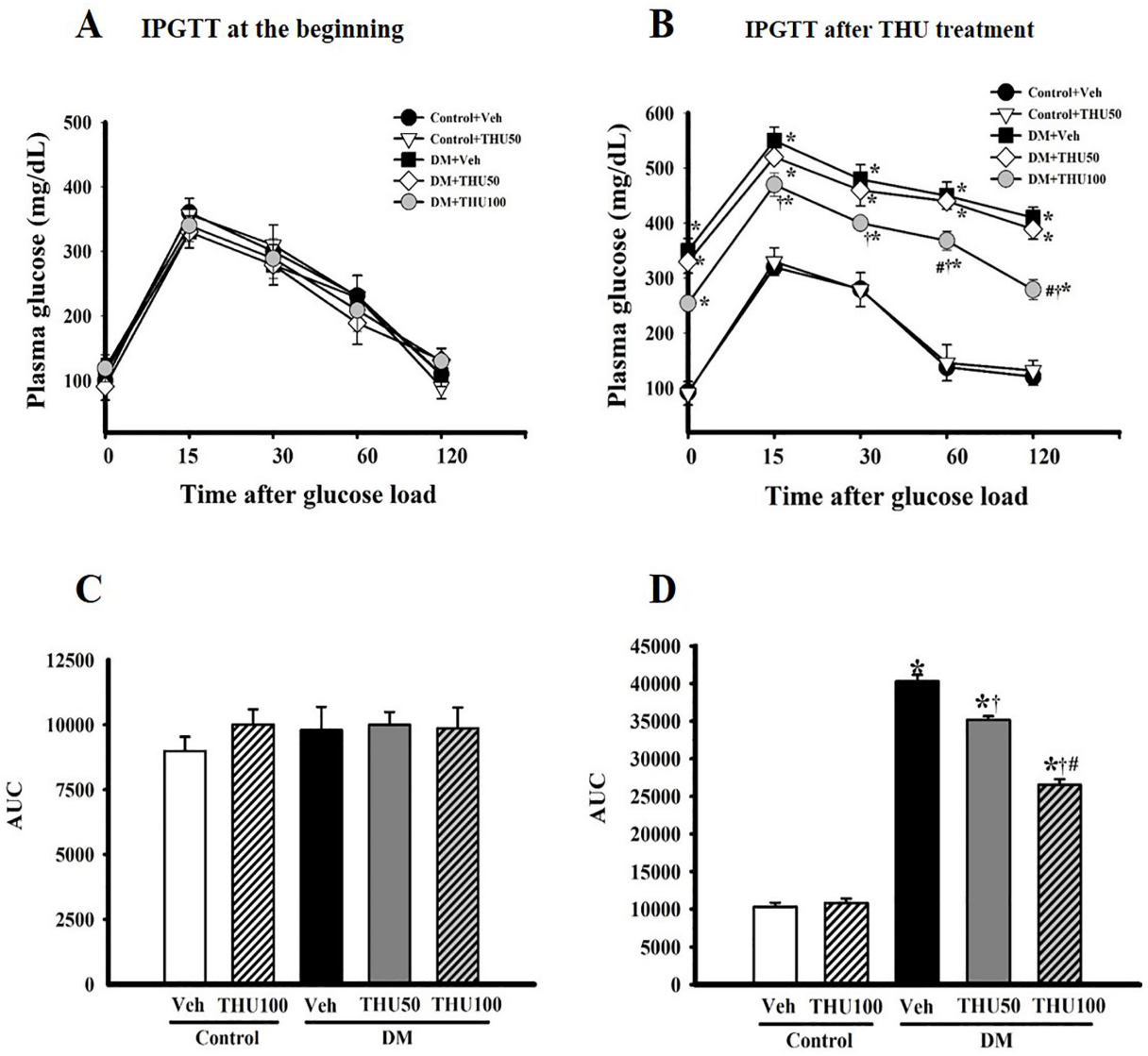

Figure 1 Effect of THU on intraperitoneal glucose tolerance test in diabetic rats before and after THU treatment. IPGTT has done at the beginning of the study (A) and IPGTT done at the end of the experiment (B). The area under the curve (AUC) was determined, (C) IPGTT done at the beginning (D) IPGTT done at the end of the study. Data are presented as mean ± SEM. *$p<0.05$ vs. normal controls; † $p<0.05$ vs. DM, and #$p<0.05$ vs.DM+THU50. N=7-8/group

(MDA), a lipid oxidation maker was found in diabetic rats ($p<0.05$ vs. normal controls; Fig. 2A). Furthermore, in carbonyl groups, a marker of protein oxidation was significantly increased in those rats when compared with normal controls ($p<0.05$; Fig. 2B). Administration with THU, especially in a high dose significantly reduced the MDA levels and protein carbonyl in plasma of diabetic rats.

Apart from oxidative stress, the antioxidant is very important for maintaining cellular redox homeostasis. Glutathione (GSH) is an intracellular antioxidant. Diabetes significantly decreased GSH levels in diabetic rats compared with normal controls ($p<0.05$; Fig. 2C). Supplementation with THU, especially at a high dose markedly ameliorated oxidative stress by increasing the blood GSH of diabetic rats.

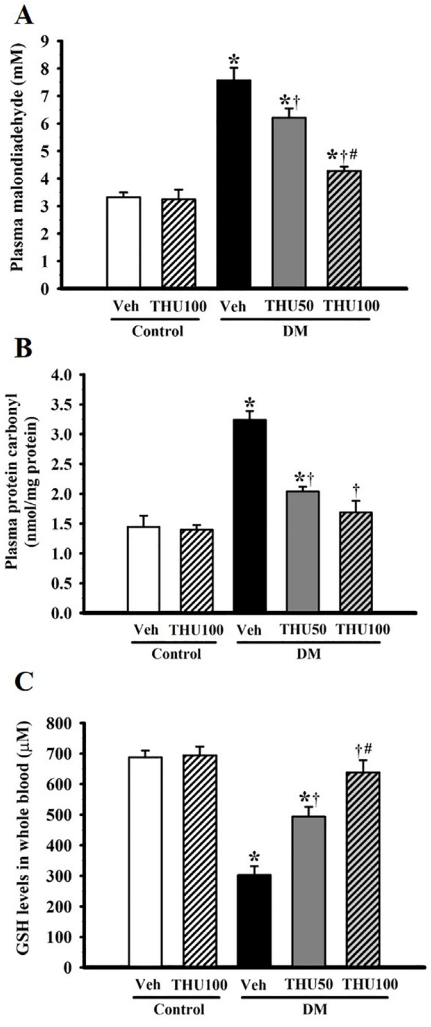

Figure 2 Effect of THU on oxidative stress and antioxidant GSH in diabetic rats. Level of lipid peroxidation marker, MDA was delectated in plasma of all experimental groups at the end of experiment (A), plasma protein carbonyl (B), and levels of GSH in whole blood (C). Data are presented as mean ± SEM. *$p<0.05$ vs. normal controls; † $p<0.05$ vs. DM, and # $p<0.05$ vs.DM+THU50. N=5-6/group

Discussion

This study demonstrated that treatment with THU for 8 weeks can effectively alleviate moderate hyperglycemia, impaired insulin sensitivity, glucose intolerance, and increased oxidative stress in rats with type 2 diabetes.

Administration of STZ and NA to young adult rats allow for the induction of type 2 diabetes. STZ is well known to cause pancreatic β-cell damage, whereas NA is administered to rats to partially protect insulin-secreting cells against STZ. Although this model is not perfectly mimic the pathogenesis of type 2 diabetes in human[2, 12], but this model is wildly accepted because it potentially produces the clinical signs and complication of type 2 diabetes in human[14]. Importantly this experimental animal model opens the opportunities for studying the diabetic drug and the interested compound on alleviating diabetic sign and complication.

In the present study, diabetic rats showed lower body weight gain at the end of the experiment when compared with normal controls. This result suggested that rats with overt diabetes lose body weight from increasing catabolism such as increased muscle wasting by protein lysis, and lipolysis. Moreover, diabetic rats presented polyuria which led to reduced body fluid consequently reduced body weight.

Under the physiologic condition, insulin plays a dominant role in body metabolism by stimulating gluconeogenesis, lipogenesis, and protein synthesis in muscle[15, 16]. Therefore, the resistance of insulin leads to alteration of body metabolism by increasing catabolism of proteins, lipids, and nucleic acids[5]. Moreover, the elevation of fasting blood glucose (FBG), insulin resistance (increased HOMA-IR), and glucose intolerance were detected in diabetic rats confirmed the disturbance of insulin homeostasis occurred in those rats.

STZ-NA-induced diabetes is associated with the initiation and propagation of ROS-induced oxidative damage to cells that can lead to failure of biological functions and ultimately cell death[11, 17]. In this study, diabetes increased lipid peroxidation as the increasing of plasma MDA. Diabetes also increased protein oxidation by increasing plasma protein carbonyl levels. This confirmed that oxidative stress occurred in the body system of diabetic rats. Moreover, the blood GSH, an intracellular antioxidant was greatly suppressed in diabetic rats, indicating STZ-NA-induced DM altered redox state in the cells.

Table 1 Effect of THU on metabolic parameters in diabetic rats

Variable	Control		DM		
	Veh	THU100	Veh	THU50	THU100
Body weight (g): at baseline	256.46 ± 2.25	252.07 ± 1.44	253.62 ± 2.25	253.91 ± 1.44	255.78 ± 1.34
Body weight (g): end of experiment	491.46 ± 1.65	489.96 ± 1.62	435.70± 1.35	470.21 ± 1.45	478.23 ± 1.77
% Change of body weight	+91.63 ± 3.63	+94.37 ± 8.49	+71.79 ± 7.66*	+85.18 ± 8.25*,†	+86.96 ± 8.38*, †
FBG (mg/dL)	93.74 ± 1.96	93.29 ± 1.70	289.93 ± 1.30*	264.12 ± 1.41*, †	224.62 ± 1.39*, †, #
Insulin (μIU/mL)	15.37 ± 0.59	15.70 ± 0.13	14.26 ± 0.36*	14.91 ± 0.55*	14.51 ± 0.25*
HOMA-IR	3.57 ± 0.02	3.38 ± 0.08	9.98 ± 0.12*	9.48 ± 0.08*, †	7.78 ± 0.11*, †, #

Data expressed as mean ± SEM. *p<0.05 as compared with normal control group; †p<0.05 as compared with diabetic group, and # p<0.05 as compared with diabetic group+THU50.N=7-8

Oral administration of THU in diabetic rats for 8 weeks prevented the loss of the body weight gain in those diabetic rats. The possible mechanism might be associated with the improvement of insulin sensitivity (reduced HOMA-IR), through the suppression of free-radicals formation. These findings agreed with previous study demonstrated that THU improved insulin sensitivity[18, 19].

The effect of THU on the reduction of hyperglycemia may be partially explained by the properties of THU contained some important functional group, such as the β-diketone moiety which possesses a strong antioxidant. Attenuation of oxidative stress is directly associated with increased insulin sensitivity by reducing the oxidation of the insulin receptors and intracellular glucose transporters[20]. Although in the present study, THU cannot increase plasma insulin levels, insulin sensitivity is improved suggesting that THU may prevent the alteration of the insulin receptor signal transduction. Our result is consistent with the previous studies that demonstrated that impaired insulin sensitivity of diabetic rats is attenuated after treatment with THU[18, 19].

The increased plasma MDA, protein carbonyl, and reduced GSH were greatly restored in diabetic rats after treatment with THU. It has been demonstrated that THU, itself, possesses a strong antioxidant property by scavenging superoxide anion (O_2^{-})[8, 10] thus preventing oxidative stress and consequently reducing cellular damages.

Accumulative result revealed that THU at dose 100 mg/kg/day exerts beneficial effects than the lower dose (50 mg/kg/day). Our result agrees with the previous studies which demonstrated that administration of THU at 100 mg/kg/day for 8 weeks provided protective effect on L-NAME-induced hypertensive rats and iron overload mice[8, 10]. This result might be explained by the concentration of THU. However, the side effect of THU in rats is found after supplementation of THU 3000 mg/kg/day in those rats[19].

In this study we did not assigned the positive group since we intended to study the effect of THU on oxidative stress and antidiabetic effect in animal model of type 2 diabetes. Moreover, good positive control such as Metformin has the different mechanism on reducing plasma glucose levels and antioxidative effect. However, using positive control might be used in our further study.

Conclusions

The results obtained from this study revealed the beneficial effect of THU on the alleviation of hyperglycemia, insulin resistance, and oxidative stress in STZ-NA diabetic rats.

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