Scientific Research Publishing

American Journal of Plant Sciences, 2017, 8, 1170-1181
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Hypoglycemic and Antioxidative Effects of *Glossogyne tenuifolia* on Streptozotocin-Nicotinamide-Induced Diabetic Rats

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How to cite this paper: Wang, R.-Y., Huang, J.-H., Wang Hsu, G.-S., Lin, W.-T. and Lu, Y.-F. (2017) Hypoglycemic and Antioxidative Effects of *Glossogyne tenuifolia* on Streptozotocin-Nicotinamide-Induced Diabetic Rats. *American Journal of Plant Sciences*, **8**, 1170-1181.
https://doi.org/10.4236/ajps.2017.85077

Received: October 12, 2016
Accepted: April 27, 2017
Published: April 30, 2017

Abstract

Glossogyne tenuifolia (GT) is the traditional herbal tea in Penghu Island, Taiwan. Recent research has shown that the active components in GT are potential inhibitors of α-glucosidase. The present study investigated that whether or not GT could improve the status of type 2 diabetes mellitus. Male Wistar rats aged eight weeks were induced to be hyperglycemic by subcutaneous injection of streptozotocin-nicotinamide (STZ-NA) and combination of high-fat diet (HFD). The animals were given GT extracts at the low dose or high dose, or the anti-diabetic drug (acarbose), in drinking water for 4 weeks. The results showed that hot water extracts from GT resulted in significantly decreases in fasting blood glucose at the 1st and 2nd weeks, fasting insulin levels at the 2nd week, 1 hour postprandial blood glucose after the starch tolerance test on Day 23 and blood glucose levels after oral glucose tolerance test (OGTT) at the 60th minute on Day 25. In addition, diabetic rats treated with GT extracts from hot water for 4 weeks displayed significantly decreased thiobarbituric acid reactive substances (TBARS) in the serum, liver and kidney, serum total cholesterol, fasting insulin levels and homeostasis model assessment for insulin resistance (HOMA-IR). Overall, these results demonstrate that the hot water extracts of GT might improve the progression of diabetes and decrease oxidative stress in STZ-NA-induced diabetic rats.

Keywords

Glossogyne tenuifolia, Blood Glucose, Antioxidative, Diabetic Rats

1. Introduction

Diabetes mellitus (DM) is currently recognized as a chronic metabolic disease with the highest rates of prevalence and global public health concern. The disease is caused by an absolute or relative lack of insulin and/or reduced insulin activity. Type 2 diabetes mellitus (T2D) is a heterogeneous disorder characterized by a progressive decline in insulin sensitivity followed by pancreatic β-cell dysfunction [1]. It is characterized by hyperglycemia and its complications like diabetic retinopathy, diabetic nephropathy and cardio vascular diseases are the most common endocrine disorder [2]. Although the leading mechanism of diabetic complications remains unclear, much attention has been given to the role of oxidative stress contributing to diabetic complications [3], including an increase in lipid peroxidation. Insulin secretion deficiency, insulin tolerance or postprandial hyperglycaemia are subject to increases in blood glucose level and cause serious damage to body systems, such as blood vessels and nerves [4] [5]. Inhibition of α-glucosidase and α-amylase would retard glucose absorption and hence decrease postprandial hyperglycaemia.

Many of the drugs currently used in DM are expensive and side effects are of serious concern [6]. Hence, natural products with perceived cost-effectiveness and no long-term side effects but which elicit better antidiabetic activities are highly desired. The health-promoting properties of some herbal teas or Chinese herbal medicines reported to be rich in antioxidants, particularly phenolics and antioxidant vitamins, can exert inhibitory capacities against α-glucosidase and exert hypoglycemic effects [7] [8] [9]. *Glossogyne tenuifolia* (GT) Cassini, also called Hsiang Ju grass, is a perennial herb with woody stems at the base which is distributed mainly in exposed coastal areas of Penghu Island, Taiwan China. It is traditionally used to make a healthy herb tea for preventing sunstroke and has a long history of being used as an antipyretic, hepatoprotective and anti-inflammatory remedy in folk medicine among local residents. Some effective components from GT have been isolated and shown to exert combinatorial anti-inflammatory and antiviral effects in production of some mediators, *i.e.*, TNF-alpha, IL-6 and IFN-gamma in activated human whole blood [10]. Its components, e.g., luteolin and luteolin-7-glucoside, play a crucial role in increasing scavenging capacity of free radicals and inhibiting the growth of hepatoma cancer cell lines [11].

Studies indicate that phenolic compounds, such as oleanolic acid, luteolin and luteolin-7-glucoside exhibited inhibitory activity on α-glucosidase [12] [13]. In our previous studies, GT extracts showed antioxidant activity demonstrated by a series of models *in vitro* and hepatoprotection *in vivo* [14], and major phenolic components, luteolin-7-glucoside and chlorogenic acid, were determined in hot water extract [14]; the latter component was recently showed to improve glucose tolerance and increase insulin sensitivity [15]. However, it is still unclear whether GT exhibits an inhibitory effect on α-glucosidase *in vitro* and hypoglycemic effect in an animal model. Hence, the present study was undertaken to investigate possible hypoglycemic and antioxidant effects of GT extracts on streptozo-

tocin-nicotinamide-induced diabetic rats.

2. Materials and Methods

2.1. Extract Preparation

Glossogyne tenuifolia (GT) Cassini (Hsiang Ju grass) was obtained and extracted as previously described [14]. Briefly, dried samples (50 g) were ground, mixed with 500 mL of deionized water and boiled for 1 hour. The aqueous mixture was centrifuged and the supernatant was filtered, the residues were then extracted again with additional 500 mL water as described above. The combined extracts from hot water were then taken and concentrated to dryness by vacuum freeze-drying to get a powder. For 50% ethanol and 95% ethanol extraction, dried samples were ground, mixed with 500 mL of individual solvents and stirred for 24 h at room temperature. The mixture was centrifuged and filtered as in the hot water extraction, and finally dried by vacuum rotary evaporator at 40˚C. The yields rates of the extracts by hot water or ethanol were similar with the results of those in previously reported [14].

2.2. Inhibition of α-Glucosidase and α-Amylase Activities *in Vitro*

The inhibitory activities of the various extracts re-dissolved in extraction solvents were subjected to assays. The inhibition for α-glucosidase from yeast and rat intestine were measured according to Kim *et al.* [16] and Oki *et al.* [17], respectively as modified by Elya *et al.* [18]. Alternatively, test samples (the extracts and acarbose) were dissolved in 0.1 M phosphate buffered saline (PBS) (pH 6.8), after filtration, 600 µL of each solution were added to 120 µL 0.2 U/mL α-glucosidase and 120 µL 0.1 M PBS (pH 6.8). The mixture was kept at 37˚C for 10 min, after preincubation, 120 µL 2.5 mM *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) was added as the substrate for 20 min, then 2 mL 0.25 M Na_2CO_3 was added to stop the reaction. The absorbance was measured at 405 nm. The rate of inhibition was calculated by the following formula: Inhibition rate (%) = $[(A_C - A_S)/(A_C - A_B)] \times 100\%$, in which A_C, A_S, and, A_B were the absorbance of control, sample, and blank, respectively. The α-amylase activities were analyzed according to Kim *et al.* [13]. In brief, the assay mixture consisted of 20 mM PBS (pH 6.9), the sample extracts (500 µL), and 500 µL 2 U/mL α-amylase type VI-B (from porcine pancreas). The mixture was preincubated for 10 minutes at 37 C, and the reaction was initiated by adding 500 µL 0.5% starch, followed by incubating the mixture again for 10 minutes at 37 C. One mL 3,5-dinitrosalicylic acid (DNS) was added to cease the reaction. The amount of glucose released in this reaction was determined by a commercially available glucose estimation kit. The concentration of the extract required to inhibit 50% of α-glucosidase or α-amylase activities under the assay conditions was defined as the IC_{50} value.

2.3. Animal Study

2.3.1. Experimental Design

Male Wistar rats weighing 170 - 190 g obtained from BioLASCO Taiwan Co.,

Ltd. were used for the study. All animals were housed singly in stainless-steel cages and fed a laboratory rodent diet (#5001, PMI Feeds, Inc., Brentwood, MO, USA), *ad libitum* for 1 week prior to experiments. The room was maintained at 22˚C ± 1˚C, 60% ± 10% RH, and with a day/night alternation of 12 h/12h. T2D were induced with subcutaneous injections of nicotinamide (NA, 230 mg/kg) and streptozotocin (STZ, Sigma, St. Louis, MO) at a dose of 65 mg/kg in ice-cold 10 mmol/L citrate buffer (pH 4.5) [19], then the animals were switched to a high fat-containing diet (40% energy from fat). A second dose of NA and STZ were administered 24 h later. Control animals received subcutaneous injections of citrate buffer only (control group). After one week, fasted rats were subjected to oral glucose tolerance test (OGTT) at a dose of 1.5 g/kg for 1 h, the rats with blood glucose more than 220 mg/dL were considered as successfully induced DM and used for the experiments. All animal experimental procedures were reviewed and approved by the Animal Experimental Committee of Fu Jen University (IACUC No. A10056).

DM rats were divided into the following treatment groups ($n = 6$ rats/group): Include the control group in experimental design. (1) Diabetes mellitus (DM) group, (2) DM with low dose GT extracts, 50 mg/kg, DML group, (3) DM with high dose GT extracts, 150 mg/kg, HML group, (4) DM with acarbose, DMA group. The GT powder and 20 acarbose (mg/kg bw) were solubilized in deionized drinking water for 4 weeks. The drinking water was freshly prepared according to the volume consumed on the previous day. Food intake and drinking water were recorded daily and body weight was recorded weekly.

2.3.2. Sample Preparation and Biomarker Analyses

On the 23th day of the experiment, rats were fasted and postprandial blood glucose was examined at one hour after starch loading test (2 g/kg) [20]. On the 25th day of the experiment, rats were fasted overnight prior to oral glucose tolerance test (OGTT) via gastric gavage using a glucose solution (1 g/kg) [21]. Blood samples were taken from the tail vein of rats at 0, 30, 60, 90, 120 and 180 min. Homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated as described by Keskin *et al.* [22], using following formula.

$$\text{HOMA-IR} = \left[\left(\text{fasting insulin } (\mu\text{U/L}) \times \text{fasting glucose } (\text{mmol/L})\right)/22.5\right].$$

At the end of the experiment, rats were euthanized in a carbon dioxide chamber; blood was withdrawn by cardiac puncture and serum was separated and stored at −80˚C (Thermo Scientific 700 Series) for later analysis of biochemical parameters. The livers, kidney and pancreas were quickly excised, rinsed in ice-cold saline (150 mM), blotted, weighed and frozen at −70˚C until analyzed as previously reported [23].

Glucose was determined by the glucose oxidase method (Randox). Serum insulin levels were analyzed by ELISA kits for rats (Mercodia Co., Sweden). The thiobarbituric acid-reactive substances (TBARS) of serum and tissue (liver, kidney and pancreas) were measured [24] using tetraethoxypropane as the standard.

2.4. Statistical Analysis

The results were expressed as means ± SD for six rats in each group. Data were analyzed by one-way ANOVA followed by testing differences between pairs by Tukey's range test. $P < 0.05$ was regarded as statistically significant. Data analysis was done using SAS version 9.3.

3. Results

3.1. Effect of Various Extracts on the Inhibitory Capacities of *α*-Glucosidase and *α*-Amylase

The inhibitory effects of GT on *α-glucosidase and α-amylase* are shown in Table 1. The HWGT had better inhibitory capacity than those of E95GT and E50GT, irrespective of the enzymes derived from *Saccharomyces cerevisiae* or rat small intestine, while the inhibitory activity (IC_{50} value) were comparable to that of the acarbose. The HWGT got the lowest IC_{50} value of *α*-glucosidase derived from rat small intestine and exhibited the highest inhibition of all GT extracts from various solvents.

3.2. Water Intakes

There was no significant difference in daily water intakes among all groups during the first and second weeks. However, the water intakes of negative control group (DM group) was higher than that of control group (Con) at the 3rd and 4th weeks, but comparable with treatment groups (DML, DMH) (data not shown).

3.3. Effects of GT Extracts on Blood Glucose and Insulin Levels

The postprandial blood glucose concentration of diabetic rats was much higher than that of that of control group by oral glucose tolerance test for 1 hour at the 0 week (initial) of experiment (272 - 280 mg/dL vs. 120 mg/dL). Figure 1 shows the glucose levels of fasting blood in diabetic rats during the experiment period. The concentration of blood glucose in rats with DM was markedly higher than that of control group. The addition of GT extracts, either at low (DML) or high dose (DMH), to the drinking water significantly decreased the blood glucose

Table 1. Inhibitory effects of *Glossogyne tenuifolia* (GT) extracted by different solvents on *α*-glucosidase and *α*-amylase[1].

Enzyme and source	E95GT[2]	E50GT	HWGT	Acarbose
α-glucosidase from	IC_{50}[3] (mg/mL)			
Saccharomyces cerevisiae	$0.78 \pm 0.25^{ab*}$	$1.06 \pm 0.35^{a*}$	$0.52 \pm 0.02^{b*}$	0.68 ± 0.05^{ab}
Rat small intestine	2.89 ± 0.36^{a}	2.66 ± 0.21^{a}	1.29 ± 0.16^{b}	$0.25 \pm 0.01^{c*}$
α-amylase from				
Porcine pancreas	NI[4]	NI	NI	0.064 ± 0.001

[1]Data are expressed as the mean ± SD (n = 3); [2]E95: 95% ethanol; E50: 50% ethanol; HW: hot water; Acarbose: positive control; [3]IC_{50}: Inhibitory concentration of 50%; [4]NI: No inhibition; [abc]Values with different superscripts in the same row are significantly different at $p < 0.05$; [*]Significantly different from corresponding rat small intestine at $p < 0.05$.

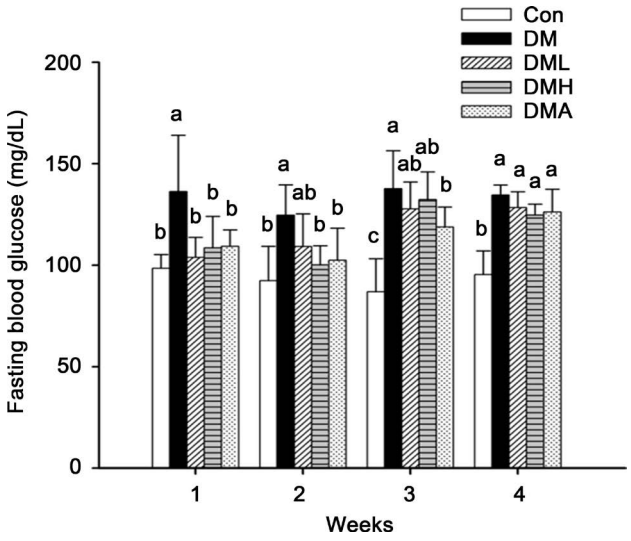

Figure 1. Effects of different dosage of GT extracts in drinking water on fasting blood glucose in diabetic rats during experimental period[1-3]. [1]Data are expressed as the means ± SD (n = 6); [2]Diabetic rats were induced by streptozotocin (STZ, 65 mg/kg) and nicotinamide (NA, 230 mg/kg); [3]Con: control; DM: diabetes mellitus; DML: diabetes mellitus with low dose *Glossogyne tenuifolia*; DMH: diabetes mellitus with high dose *Glossogyne tenuifolia*; DMA: diabetes mellitus with acarbose; GT: *Glossogyne tenuifolia*. [abc]Values with various letters are significantly different at $p < 0.05$.

concentration of rats (average ca. 19% - 24% lower), and the lowering effect was similar between DML and DMH groups for the first and second weeks. The blood glucose of positive group (DMA) decreased from the first week to third week. However, the blood glucose was comparable in all DM rats, irrespective of giving GT extracts or acarbose at the end of the experiment.

Although rats fed DMH or DMA had lower tendency toward insulin concentration than that of DM group, the insulin concentration DML rats was lower than that of DM group (Table 2). The calculated HOMA-IR was higher in DM rats than that of control group; the addition of GT to drinking water could reduce HOMA-IR. Especially, the HOMA-IR of DMH rats was significantly lower than that of DM rats at both 2 and 4 weeks.

3.4. Effects of GT Extracts on Postprandial Blood Glucose and Oral Glucose Tolerance Test (OGTT)

Postprandial blood glucose level of DM group was markedly increased by double time compared to that of control group at one hour after starch tolerance test (223.7 ± 38.1 vs. 110.7 ± 20.7 mg/dL) on the 23th day (Figure 2). The addition of low dose GT extracts in DML and DMA groups significantly decreased the blood glucose concentration of rats (average 22.5% and 22.6% lower, respectively). Blood glucose levels by OGTT in diabetic rats being administered different dosages of GT extracts at day 25 are shown in Figure 3. It was observed that administration of glucose to animals induced to postprandial hyperglycemia, especially in rats with DM. Rats submitted to GT-containing water (DML and DMH) showed a significant decrease of glycemia by 19% - 26%, one hour after ingestion of

glucose as compared to DM rats. Thereafter, the postprandial anti-hyperglycemic effect was maintained only in DMA rats. The areas under the curve (AUC) were markedly increased in DM group compared to that of control group; the addition of GT to drinking water (both DML and DMH group) significantly reduced AUC as compared to DM group (Figure 4).

3.5. Effects of GT Extracts on Lipid Peroxidation of DM Rats

At the end of experiment, the rats with DM showed significantly increased lipid peroxidation (TBARS) by 25% - 144% of the control group, irrespectively of in

Table 2. Effects of different dosage of GT extracts in drinking water on fasting blood glucose, insulin and HOMA-IR levels in diabetic rats after 2 and 4 weeks[1,2].

Parameters	Groups[3]				
	Con	DM	DML	DMH	DMA
2 weeks					
Glucose (mg/dL)	92.4 ± 16.9^{b}	124.7 ± 14.9^{a}	109.3 ± 16.1^{ab}	100.3 ± 9.32^{b}	102.4 ± 15.8^{b}
Insulin (μg/L)	0.31 ± 0.20^{b}	0.45 ± 0.08^{a}	0.31 ± 0.06^{b}	0.33 ± 0.08^{ab}	0.33 ± 0.09^{ab}
HOMA-IR[4] (mmol·mU/L·L)	2.07 ± 1.77^{b}	4.07 ± 0.77^{a}	3.29 ± 0.63^{ab}	2.59 ± 0.73^{b}	2.48 ± 0.77^{b}
4 weeks					
Glucose (mg/dL)	95.4 ± 11.7^{b}	134.7 ± 4.8^{a}	128.4 ± 7.8^{a}	124.7 ± 5.4^{a}	126.3 ± 11.2^{a}
Insulin (μg/L)	0.77 ± 0.37^{b}	1.50 ± 0.35^{a}	0.74 ± 0.29^{b}	0.91 ± 0.45^{b}	0.73 ± 0.44^{b}
HOMA-IR (mmol·mU/L·L)	6.02 ± 3.33^{b}	13.82 ± 3.50^{a}	9.30 ± 5.17^{ab}	8.43 ± 4.65^{b}	8.21 ± 4.81^{b}

[1]Diabetic rats were induced by streptozotocin (STZ, 65 mg/kg) and nicotinamide (NA, 230 mg/kg); [2]Data are expressed as the mean ± SD (n = 6); [3]Con: control; DM: diabetes mellitus; DML: diabetes mellitus with low dose *Glossogyne tenuifolia*; DMH: diabetes mellitus with high dose *Glossogyne tenuifolia*; DMA: diabetes mellitus with acarbose; GT: *Glossogyne tenuifolia*; [4]HOMA-IR: homeostasis model assessment for insulin resistance; [ab]Values with different superscripts in the same row are significantly different at $p < 0.05$.

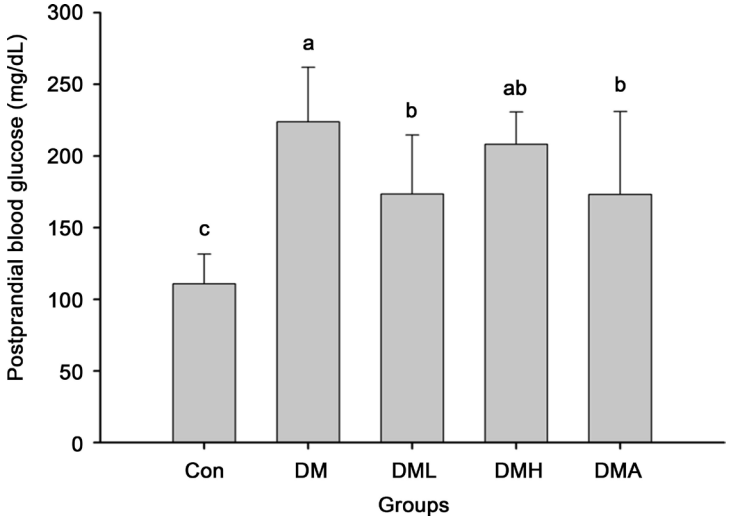

Figure 2. The postprandial blood glucose concentration after starch loading test on the 23th day[1-2]. [1]Data are expressed as the means ± SD ($n = 6$); [2]Diabetic rats and abbreviation: see Figure 1; [abc]Values with various letters are significantly different at $p < 0.05$.

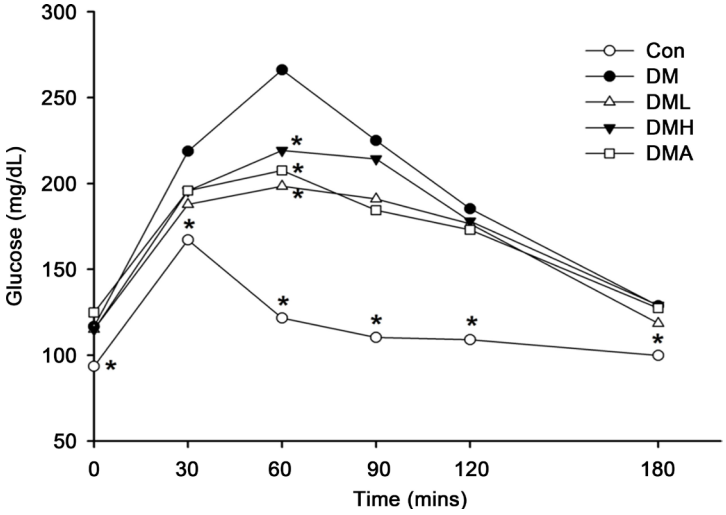

Figure 3. Blood glucose levels by oral glucose tolerance test (OGTT) in diabetic rats being administered different dosages of GT extracts in drinking water on day 25[1-2]. [1]Data are expressed as the means ± SD (n = 6); [2]Diabetic rats and abbreviation: see **Figure 1**; *Significantly different from the DM group at $p < 0.05$.

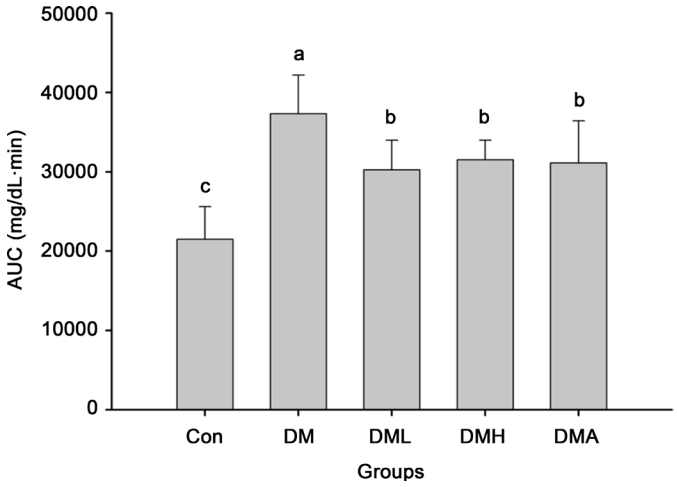

Figure 4. Areas under the curve (AUC) of blood glucose concentration by oral glucose tolerance test (OGTT) in diabetic rats being administered different dosages of GT extracts in drinking water on Day 25[1-2]. [1]Data are expressed as the means ± SD (n = 6); [2]Diabetic rats and abbreviation: see **Figure 1**; [abc]Values with various letters are significantly different at $p < 0.05$.

Table 3. Effects of different dosage of GT extracts in drinking water on serum, liver, kidney and pancreas TBARS in diabetic rats after 4 weeks[1].

Parameters	Groups[3]				
	Con	DM	DML	DMH	DMA
Serum TBARS[2] (nmol/mL)	3.80 ± 0.67[c]	8.26 ± 1.50[a]	6.67 ± 0.66[b]	7.23 ± 0.68[b]	6.99 ± 0.59[b]
Liver TBARS (nmol/g liver)	12.0 ± 2.18[c]	26.5 ± 3.67[a]	19.4 ± 6.08[b]	21.5 ± 2.39[b]	18.5 ± 4.72[b]
Kidney TBARS (nmol/g kidney)	72.5 ± 6.00[b]	90.1 ± 13.9[a]	74.6 ± 9.18[b]	75.3 ± 4.43[b]	86.2 ± 19.3[ab]
Pancreas TBARS (nmol/g pancreas)	12.7 ± 2.07[b]	31.0 ± 16.2[a]	28.4 ± 15.8[ab]	19.2 ± 11.3[ab]	18.3 ± 8.14[ab]

[1]Diabetic rats and abbreviation: see **Table 2**; [2]TBARS: thiobarbituric acid-reactive substances; [abc]Values with different superscripts in the same row are significantly different at $p < 0.05$.

serum or tissue (liver, kidney and pancreas) (Table 3). In comparison with DM group, DM rats treated with GT extracts either at low dose (DML) or high dose (DMH) exhibited significantly lower TBARS in serum, liver and kidney, while the pancreas TBARS were comparable with that of control group.

4. Discussion

Retarding of glucose absorption by inhibition of carbohydrate-hydrolysing enzymes, such as α-glucosidase and α-amylase is one of the therapeutic approaches to decreasing postprandial hyperglycaemia [16]. Therefore, many studies have been conducted to find effective inhibitors of α-glucosidase and α-amylase from natural materials and to develop physiological functional food for treating DM [7] [25]. Our results show that inhibition ability of hot water extract (HWGT) for α-glucosidase from yeast were better than that of rat small intestine ($p = 0.013$). Moreover, inhibition ability of HWGT was better than that of ethanol extracts either from 95% or 50% ethanol. Another study showed that acarbose is a good inhibitor for α-glucosidase [26]; our experiment showed similar results and inhibition capacity for α-amylase. Type-I α-glucosidase, derived from yeast, is usually of higher purity, while α-glucosidase, derived from rat small intestine, Type-II, mimics mammals more [27]. Inhibition ability of acarbose for type-II α-glucosidase is superior to that of type-I [17]; similar results were found in our experiment.

Administration of both streptozotocin (STZ) and nicotinamide (NA) has been shown to induce an experimental T2D model in rats [28]. STZ induces DM by destroying pancreatic β-cells which produce insulin for the body. Nitric oxide induced by STZ may play a role in the cytotoxic action of STZ in pancreatic β-cells. Nicotinamide has been shown to mediate a protective effect against cellular damage induced by different toxic agents, including STZ [29]. It is easy to induce insulin resistance and glucose intolerance in rats fed a high fat diet [30]. STZ combined high fat diet induced DM model would lead to animals with insulin resistance and stable high blood that mimic the syndrome of human T2D [31]. The hypoglycemic effect of HWGT might be at least partly attributed to its active compounds such as chlorogenic acid and luteolin-7-glucoside, as indicated in previous analyses [14].

HOMA-IR is one of the markers for evaluating insulin resistance [32]; our results showed that DM group had higher insulin levels and HOMA-IR values than that of control group, implying that high fat diet combined with STZ-NA can successfully induce a model of insulin resistance. In addition, HWGT significantly lowered insulin levels and HOMA-IR values at weeks 2 and 4 of the experiment. It may be postulated that chlorogenic acid, one of the active components in HWGT, can stimulate insulin sensitivity and improve insulin resistance [15].

GT extracts, either with hot water or 95% ethanol, exhibit better antioxidant capacity and reducing powder than that of GT extract with cold water [33]. These results were comparable with our previous experiment. Furthermore, GT

extracts with hot water or 95% ethanol exhibit similar antioxidant capacity *in vitro*, *i.e.*, DPPH radical scavenging activity, reducing power and total antioxidant capacity. [14]. Rats with DM showed a significant increase in lipid peroxidation (TBARS) in various tissues compared to the control group. However, DM rats treated with GT extracts either at low dose (DML) or high dose (DMH) exhibited significantly lower TBARS in serum, liver and kidney and pancreas TBARS values were comparable with that of control group. The phenolic compounds, chlorogenic acid and luteolin-7-glucosidein hot water extract of GT might play an important role in antioxidant capacity in this experiment.

5. Conclusion

To the best of our knowledge, the results of the present study demonstrate, for the first time, that *G. tenuifolia* exerts anti-diabetic effects and antioxidative capacity in STZ-NA-induced T2D rats by regulating blood glucose levels and lipid peroxidation. This regulation may involve the retardation of postprandial hyperglycaemia and decrease fasting insulin levels and HOMA-IR. Thus, the present results demonstrate the potential therapeutic benefit of *G. tenuifolia* in regulating blood glucose levels in diabetic patients. Additional long-term studies are needed to confirm this beneficial effect.

Acknowledgements

The study was supported by a grant, 101-2313-B-030-003-MY3, from the Ministry of Science and Technology, Taiwan.

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