Tropical Journal of Pharmaceutical Research April 2020; 19 (4): 829-835
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v19i4.22

Original Research Article

Anti-diabetic effect of the polyphenol-rich extract from *Tadehagi triquetrum* in diabetic mice

Xiaowan Lin[1,2], Xinxin Zhou[1,2], Wanying Sun[2], Lu Zhang[2], Caiyun Zhang[2], and Xiaopo Zhang[2]*

[1]Pharmaceutical Engineering Technology Research Center, College of Pharmacy, Harbin University of Commerce, Harbin 150076, [2]Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Key Laboratory for Research and Development of Tropical Herbs, School of Pharmacy, Hainan Medical University; Haikou 571199, China

For correspondence:* ***Email:* *z_xp1412@163.com*

Sent for review: 12 November 2019 *Revised accepted: 30 January 2020*

Abstract

Purpose: *To clarify the diabetes-reducing abilities of the polyphenol-rich extract from Tadehagi triquetrum (HC) in diabetic ob/ob mice.*
Methods: *Aerial parts of T. triquetrum were extracted under reflux and partitioned by n-butanol to generate HC. The effects of HC consumption on blood glucose and lipids, insulin resistance, and liver glucose metabolism were evaluated in vivo. The main compounds of HC were tested for their effects on stimulating glucose consumption and uptake by HepG2 hepatocytes and C2C12 myotubes.*
Results: *After HC treatment, body fat, subcutaneous fat, and epididymal fat masses decreased ($p < 0.05$), while mean daily food intake was unaffected. HC (200–400 mg/kg) decreased fasting blood glucose, glycosylated serum protein (GSP), and glycosylated hemoglobin (HbAlc); it also lowered hyperinsulinemia, improved oral glucose tolerance, and reduced hyperlipidemia and liver fat content ($p < 0.05$). HC treatment markedly elevated liver glycogen content and activity of hepatic glucokinase and pyruvate kinase ($p < 0.05$). Eight polyphenols were isolated from HC, six of which potently stimulated glucose consumption and uptake in vivo.*
Conclusion: *HC has potent antidiabetic activities. Polyphenols are the main compounds accounting for these effects. Chronic oral administration of HC may be an alternative therapy for managing diabetes, but this has to be subjected first to clinical studies.*

Keywords: *Tadehagi triquetrum, Diabetes, Phenylpropanoid glucosides, Pyruvate kinase, Glucokinase*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Type 2 diabetes (T2DM) is prevailed throughout the world. Hyperglycaemia and insulin resistance (IR) are main characteristics of T2DM [1]. Although multiple antidiabetic medicines are currently available, many are associated with undesirable side effects [2,3]. Consequently, there is a tremendous need for developing effective antidiabetic drugs with fewer side effects. The flowering plant *Tadehagi triquetrum* is a herbal remedy used for inflammation and liver disease [4,5]. In South China, people usually boil the aerial parts of *T. triquetrum* in

water and drink it as a tea called “Hulu tea.”

Previous chemical studies identified phenolic compounds and terpenoids as the major constituents of *T. triquetrum.* Recent investigations have revealed that phenolic compounds, especially phenylpropanoid glucosides, in this medicinal plant exhibit potent lipid- and glucose-decreasing effects *in vitro* [6-8]. However, the antidiabetic effects of *T. triquetrum* aerial parts have not been heretofore tested *in vivo*. In this study, the diabetes-reducing effects of the polyphenol-rich fraction from *T. triquetrum* aerial parts (HC) were tested in diabetic *ob/ob* mice. The antidiabetic ability of HC was evaluated. The main active compounds of HC were also examined to determine their role in the glucose-lowering abilities.

EXPERIMENTAL

Plant materials

T. triquetrum was collected from Changliu in Haikou, Hainan Province, in July 2018, and authenticated by professor Nian-Kai Zeng. After identification, a sample (no. HC-07-2018) was deposited in herbarium at Hainan Medical University.

Extraction and isolation

Dried stems and leaves of *T. triquetrum* were extracted using 70:30 ethanol:distilled water as a solvent. The resulting crude extract was dissolved in pure water and partitioned with dichloromethane to eliminate lipophilic compounds. The residue was further extracted using n-butanol to obtain HC, after concentration. HC (150 g) was isolated using a column of silica gel with a gradient solvent of chloroform-methanol to produce 6 fractions (Fractions A-F). Fraction B was processed in an additional step using the gel column, and methanol was used as the eluent to purify the compounds. The fractions obtained in this manner were further separated by high-performance liquid chromatography (HPLC) using a mixed solvent (methanol:water 35:65, v/v) and a semipreparative HPLC column, which yielded compound **6** (71.0 mg). Fraction C was isolated using the same process as for Fraction B and then purified using HPLC (with methanol:water 35:65, v/v) to generate compounds **7** (124.5 mg) and **8** (255.4 mg). Fraction D was prepared in the same manner using a gel column and HPLC (with methanol:water 42:58 v/v), generating compounds **3** (34.0 mg)**, 4 (**124.0 mg)**,** and **5** (55.0 mg). Fraction E was isolated and separated in the same way as Fraction D and purified using HPLC (with methanol:water 30:70, v/v) as above, yielding compounds **1** (20.5 mg) and **2** (30.0 mg).

Animals and experimental design

All animal experiments were performed in accordance with the animal ethics committee of Hainan Medical University (reg. no. 201706017/HMU). A total of 36 male 12-week-old mice, including 30 *ob/ob* mice and 6 C57BL/6J mice, were purchased from a qualified animal feeding company. The mice were maintained under humidity-controlled conditions with a 12-hour/12-hour light/dark cycle. They were feed *ad libitum* for 7 days and then randomly separated into six groups. Mice in the normal control (C57BL/6J mice) and negative control (vehicle only, *ob/ob* mice) were administered equivalent volumes of distilled water orally. Metformin (200 mg/kg) was applied as the positive and given daily via oral gavage, and the HC groups were administered HC daily (100, 200, or 400 mg/kg) via the same route.

The body mass of each mouse was measured weekly using a balance. Feed intake of each mouse was also recorded. After 4 weeks, all mice were fasted overnight. The next morning, their body mass was measured, and the animals were then anesthetized using intraperitoneal chloral hydrate (400 mg/kg). Approximately 0.5 mL whole blood was then withdrawn from the abdominal aorta. The animals were subsequently euthanized, after which we measured their liver, subcutaneous fat, and epididymal fat masses. Biological indicators (except blood insulin) were evaluated using commercial kits, following the manufacturer's recommendations. Serum insulin levels were quantified using a radioimmunoassay kit.

Oral glucose tolerance test

On the twenty-four days after initiating study, each mouse underwent an oral glucose tolerance (OGTT) using oral gavage of glucose (2 g/kg), as described previously [9]. Blood was obtained from tail-vein at 0, 0.5, 1.0, 1.5, and 2.0 hours after glucose administration. The glucose content was checked immediately after withdrawal using a Roche glucose meter (ACCU-CHEK Active, Roche).

Cell culture, and glucose consumption and uptake assay

The C2C12 myotube and HepG2 hepatocyte cell lines were used for *in vitro* assessment of the individual HC compounds. The origin, culturing, and handling of these cell lines have been

previously described [9,10]]. Briefly, both cells were cultured in DMEM containing 10% fetal bovine serum at a certain temperature and a fixed concentration of carbon dioxide as previous reports [9,10]. To differentiate cells, the culture medium was changed into DMEM possessing 2% horse serum. After incubation for 12 h, cells were cultured with 10 μM of each compound.

Glucose consumption and uptake assay were performed as described previously [10]. Glucose consumption was investigated on HepG2 cells. The cells were cultured with DMEM possessing HC compounds. After incubation for 24 h, the glucose content in the medium was tested according to glucose kit. Glucose uptake assay was conducted on C2C12 myotubes. Cells were cultured with the medium containing 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) and HC compounds or insulin (100 nM). After 12 h, the medium was moved away and C2C12 myotubes were cleaned by phosphate-buffered saline (PBS) for two times. After this step, the C2C12 myotubes were processed by scrapping out and then transferring. The contents of 2-NBDG uptake by C2C12 myotubes were tested according to reported method.

Statistical analysis

Data are displayed as mean ± standard error of the mean. Groups were compared using one-way analysis of variance followed by Dunnett's t-tests. Statistical analysis was performed using SPSS 22.0 software.

RESULTS

HC reduced mouse body mass

At 4 weeks, all groups of *ob/ob* mice exhibited a considerably higher body mass than normal control mice (Figure 1 a). Body mass gain from baseline to 4 weeks was also considerably more in *ob/ob* mice than in normal mice (Figure 1 b). There was a dose-dependent reduction in body mass gain in the HC-treated mice (Figure 1 b). Liver, subcutaneous and epididymal fat masses were apparently reduced with 400 mg/kg HC (Figure 1 d – f). Feed intake showed no significantly different between any of the groups (Figure 1 c).

HC decreased blood glucose

At 4 weeks, animals exhibited higher fasting blood glucose (FBG) (Figure 2 a), greater increase in FBG (Figure 2 b), HbAlc (Figure 2 c), and GSP (Figure 2 d), compared with normal mice. HC treatment was associated with reductions in FBG, HbAlc, and GSP (Figure 2 a – d). The effectiveness of HC (400 mg/kg) for decreasing blood glucose was similar to that observed with metformin. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) displayed unimportant differences between any of the groups of *ob/ob* mice, suggesting that the beneficial effects of HC were not associated with liver toxicity (Figure 3).

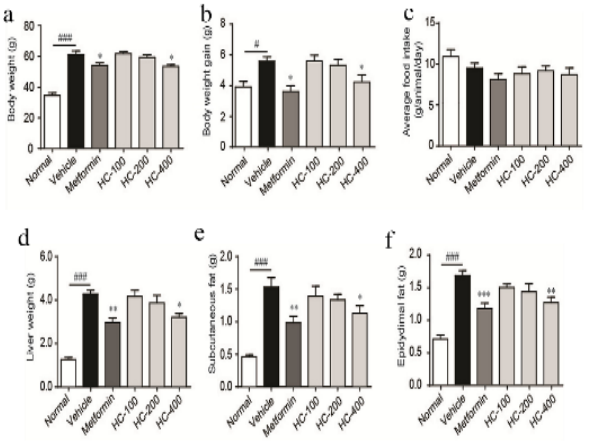

Figure 1: Effect of HC on reducing body mass. Body mass (a), liver mass (d), subcutaneous fat mass (e), and epididymal fat mass (f) were measured at 4 weeks, and body mass gain (b) and mean feed intake (c) during the 4-week period were calculated (n = 6); $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$, normal compared with vehicle; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, metformin and HC compared with vehicle

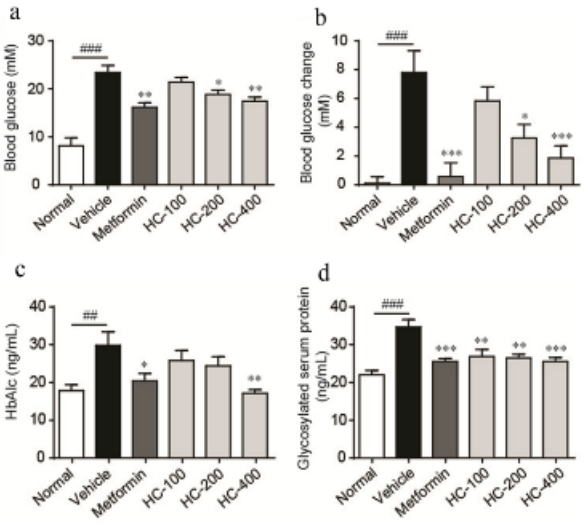

Figure 2: Effect of HC on reducing hyperglycemia at 4 weeks. (a) serum levels of glucose, (b) changes in serum levels of glucose, (c) serum HbAlc, and (d) GSP (n = 6); $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, normal compared with vehicle; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, metformin and HC compared with vehicle

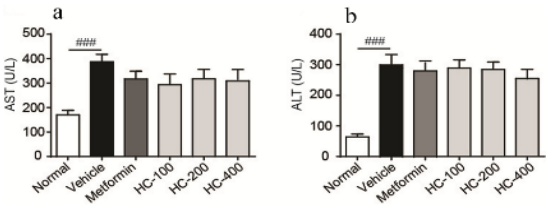

Figure 3: Effects of HC on hepatic toxicity. (a) Serum aspartate aminotransferase (AST) and (b) alanine aminotransferase (ALT) (n = 6); $^{###}p < 0.001$, normal compared with vehicle

HC alleviated insulin resistance

At 4 weeks, *ob/ob* mice had significantly higher fasting serum insulin and higher homeostasis model assessment of IR index values (reflecting significant IR), compared with normal control mice (Figure 4 a and b). Both parameters were improved in the HC (200 - 400 mg/kg) groups. On day 24, *ob/ob* mice also exhibited worse OGTT results, compared with normal mice (Figure 4 c and d). Oral glucose tolerance was markedly raised by HC or metformin, as exemplified by improvements in blood glucose, as well as area under the glucose curve ($AUC_{glucose}$), during OGTT.

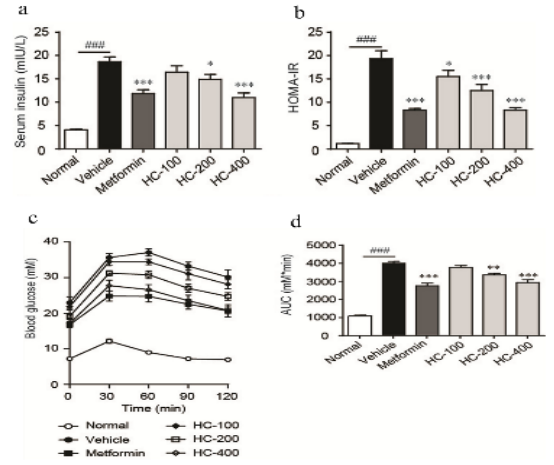

Figure 4: Effects of HC on reducing insulin levels and resistance and improving oral glucose tolerance. (a) Fasting serum insulin levels; (b) homeostasis model assessment of IR (HOMA-IR) indices; (c) blood glucose during oral glucose tolerance test; and (d) glucose area under the glucose curve ($AUC_{glucose}$) (n=6); $^{###}p < 0.001$, normal compared with vehicle; $^{**}p < 0.01$, $^{***}p < 0.001$, metformin and HC compared with vehicle

HC ameliorated hyperlipidemia

The lipid-lowering effects of HC were tested by measuring triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c). At 4 weeks, serum TG, TC, and LDL-c and liver TC and TG were higher in *ob/ob* mice than in normal mice (Figure 5). Treatment with HC (200 mg/kg and 400 mg/kg) reduced serum TG, TC, and LDL-c, as well as liver TC and TG (Figure 5). HC (400 mg/kg) appeared to be slightly more effective that in metformin for lowering serum and liver lipids. Serum HDL-c showed no significantly different between any of the groups (Figure 5 d).

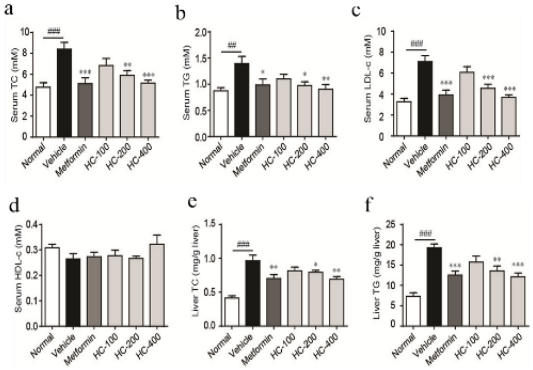

Figure 5: Effect of HC on reducing blood and liver lipids (a-d). (a) Serum total cholesterol (TC); (b) serum triglycerides (TG); (c) low-density lipoprotein cholesterol (LDL-c); (d) high-density lipoprotein cholesterol (HDL-c); liver TC (e); and liver TG (f) (n = 6); $^{##}p < 0.01$, $^{###}p < 0.001$, normal compared with vehicle; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, metformin and HC compared with vehicle

HC enhanced hepatic glycolysis and stimulated glycogen production

Liver abnormal glucose often leads to raised blood glucose levels [11]. Compared with C57BL/6J animals, *ob/ob* mice had significantly lower hepatic pyruvate kinase and glucokinase activity (Figures 6 b and c), although liver glycogen content did not significantly differ between *ob/ob* and normal mice (Figure 6 a).). Liver glycogen, as well as pyruvate kinase and glucokinase activity, increased with HC treatment (Figure 6).

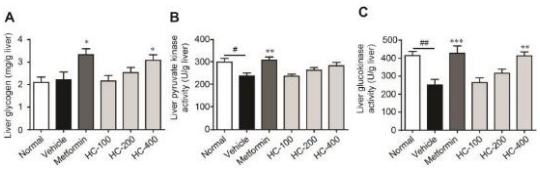

Figure 6: Effects of HC on increasing hepatic glycogen and glycolytic enzyme activity. (a) Liver glycogen content, (b) liver pyruvate kinase activity, and (c) liver glucokinase activity (n = 6); $^{##}p < 0.01$, $^{###}p < 0.001$ normal compared with vehicle; $^{**}p < 0.01$, $^{***}p < 0.001$, metformin and HC compared with vehicle

Identification of eight compounds by analyzing nuclear magnetic resonance spectral data

By comparing our phytochemistry results with reported nuclear magnetic resonance (NMR) spectroscopic data and by comparing the Thin Layer Chromatography results with those from reference compound studies, the eight compounds were identified as follows: 6-O-(*E*)-p-hydroxy-cinnamoyl-β-D-glucose (compound **1**), 6-O-(*E*)-p-hydroxy-cinnamoyl-α-D-glucose (compound **2**), tadehaginoside A (compound **3**), rutin (compound **4**), tadehaginoside D (compound **5**), tadehaginoside (compound **6**), kaempferol-3-O-rutinoside (compound **7**), and kaempferol-3-O-β-D-galactopyranosyl(6-1)-α-L-rhamnopyranoside (compound **8**) [7,12]. ^{1}HNMR spectra and structures of the eight compounds are shown in Figure 7.

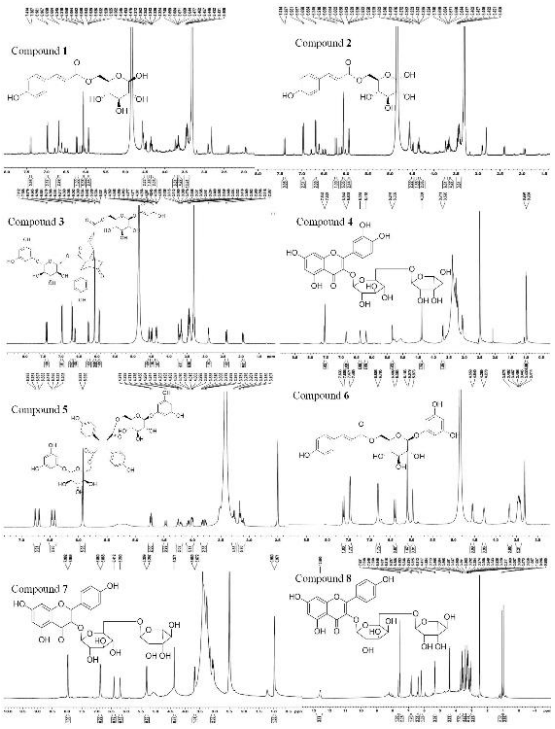

Figure 7: ^{1}HNMR spectra and structures of compounds **1** to **8** isolated from HC

Phenylpropanoid glucosides are the main active constituents of HC

Phytochemical analysis revealed that HC contained high proportions of polyphenols. To verify whether polyphenols are the active compounds of HC responsible for the observed antidiabetic effects, the eight compounds were evaluated *in vitro*. Their glucose-lowering activity was tested in HepG2 hepatocytes and C2C12 myotubes at a concentration of 10 μM. As shown in Figure 8, six polyphenols (compounds **3**–**8**), particularly compound **4**, increased glucose consumption by hepatocytes, suggesting that polyphenols are major contributors to the antidiabetic effect of HC.

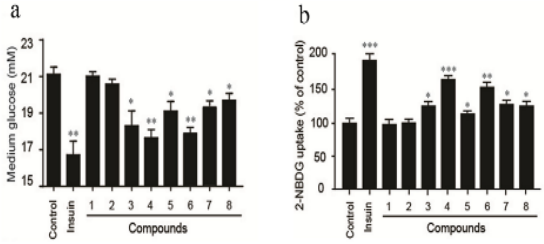

Figure 8: Hypoglycemic effect of the HC compounds. (a) Glucose consumption by HepG2 hepatocytes (reflected by reduced medium concentrations of glucose) and (b) Glucose uptake in C2C12 myotubes; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

DISCUSSION

T2DM often require long-term medications to control blood glucose levels. Dietary interventions are a simple, risk-free option for diabetes management. Dietary interventions have gained increasingly more attention from both researchers and patients. In the current study, the polyphenol-rich extract HC possessed potent diabetes-reducing effects in *ob/ob* mice. Four-week treatment with HC decreased FBG and prevented blood glucose increases dose-dependently. The efficacy of HC (400 mg/kg) for decreasing blood glucose was similar with that of metformin (200 mg/kg). Serum GSP and HbAlc levels, which are indicators of diabetes in the previous 1-3 weeks or 2 months, respectively, were also significantly decreased with HC, indicating that treatment with HC could maintain blood glucose levels over a prolonged period of time.

Polyphenol-rich extracts from various medicinal plants have documented beneficial effects in treating T2DM [13,14]. This area has attracted much research interest from both pharmacologists and chemists for many years and has been the subject of excellent reviews [15-17]. The hypoglycemic effects of phenolic compounds in HC have been investigated. Many of these have unique structures and obvious biological activities and some have shown significant effects, with potentially important roles in the treatment of diabetes [18,19].

Previously, phenylpropanoid glucosides have been extracted from the whole plants of *T. triquetrum,* some of which have been demonstrated to improve glucose consumption

by HepG2 and C2C12 cells [7, 8]. Polyphenols, and especially phenylpropanoid glucosides, may be the principal active components of HC for treating diabetes. To assess this, the glucose-lowering activity of the eight main compounds were assessed. Of these, six compounds (**3**–**8**), particularly compounds **5** and **6**, exhibited significant ability in glucose consumption and uptake *in vitro*. These data suggest that these polyphenols have glucose-lowering effects and may be the agents responsible for the antihyperglycemic activity of HC. Thus, phenylpropanoid glucosides isolated from HC could be natural compounds leading to the development of new agents for treating or curing T2DM.

CONCLUSION

HC, the n-butanol extract of *T. triquetrum* aerial parts, exhibits potent antidiabetic activity in diabetic mice. Polyphenols are the main bioactive compounds accounting for these antidiabetic effects. Chronic oral administration of HC might be an alternative therapy for management diabetes.

DECLARATIONS

Acknowledgement

This work was supported financially by the National Natural Science Foundation of China (no. 81560696).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Nankar R, Prabhakar PK, Doble M. Hybrid drug combination: Combination of ferulic acid and metformin as anti-diabetic therapy. Phytomed 2017; 37: 10-13.*
2. *Hostenkamp G, Fischer KE, Borch-Johnsen K. Drug safety and the impact of drug warnings: An interrupted time series analysis of diabetes drug prescriptions in Germany and Denmark. Health Policy 2016; 120(12): 1404-1411.*
3. *Thangavel S, Yoshitomi T, Sakharkar MK, Nagasaki Y. Redox nanoparticle increases the chemotherapeutic efficiency of pioglitazone and suppresses its toxic side effects. Biomater 2016; 99: 109-123.*
4. *Kalyani GA, Ramesh CK, Krishna V. Hepatoprotective and Antioxidant Activities of Desmodium Triquetrum DC. Indian J Pharm Sci 2011; 73(4): 463-466.*
5. *Kim KH, Kim S, Jung MY, Ham IH, Whang WK. Anti-inflammatory phenylpropanoid glycosides from Clerodendron trichotomum leaves. Arch Pharm Res 2009; 32(1): 7-13.*
6. *Wang S, Zhang X, Li X, Liu Q, Zhou Y, Guo P, Dong Z, Wu C. Phenylpropanoid glucosides from Tadehagi triquetrum inhibit oxLDL-evoked foam cell formation through modulating cholesterol homeostasis in RAW264.7 macrophages. Nat Pro Res 2019;33(6): 893-896.*
7. *Zhang X, Chen C, Li Y, Chen D, Dong L, Na W, Wu C, Zhang J, Li Y. Tadehaginosides A-J, Phenylpropanoid Glucosides from Tadehagi triquetrum, Enhance Glucose Uptake via the Upregulation of PPARγ and GLUT-4 in C2C12Myotubes.J Nat Prod 2016; 79(5): 1249-1258.*
8. *Zhang X, Wang S, Li Y, Zhao D, An N, Wu J, Zhang T, Wu C, Li Y. Tadehaginoside modulates lipogenesis and glucose consumption in HepG2 cells. Nat Prod Res 2015; 29(24): 2287-2290.*
9. *Wu C, Zhang X, Zhang X, Luan H, Sun G, Sun X, Wang X, Guo P, Xu X. The caffeoylquinic acid-rich Pandanus tectorius fruit extract increases insulin sensitivity and regulates hepatic glucose and lipid metabolism in diabetic db/db mice. J Nutr Biochem 2014; 25(4): 412-419.*
10. *Wu C, Luan H, Wang S, Zhang X, Wang R, Jin L, Guo P, Chen X. Modulation of lipogenesis and glucose consumption in HepG2 cells and C2C12 myotubes by sophoricoside. Molecules 2013; 18(12): 15624-15635.*
11. *Parlakgul G, Hotamisligil GS. Type I interferons interfere with liver glucose metabolism. Cell Metab 2017; 25(6): 1211-1212.*
12. *Wu J, Ma G, Li H, Wu C, Tan Y, Zhang T, Chen F, Guo P, Zhang X. Chemical constituents with antihyperlipidemic activities from Desmodium triquetrum. 2014; Chin Herb Med 2014; 6(4): 324-327.*
13. *Islam MS. Effects of the aqueous extract of white tea (Camellia sinensis) in a streptozotocin-induced diabetes model of rats. Phytomedicine 2011; 19: 25-31.*
14. *Sobeh M, Mahmoud MF, Abdelfattah MAO, El-Beshbishy HA, El-Shazly AM, Wink M. Hepatoprotective and*

hypoglycemic effects of a tannin rich extract from Ximenia americana var. caffra root. Phytomedicine 2017; 33: 36-42.

15. *Xiao JB, Högger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. Curr Med Chem 2015; 22(1): 23-38.*
16. *Dragan S, Andrica F, Serban MC, Timar R. Polyphenols-rich natural products for treatment of diabetes. Curr Med Chem 2015; 22(1): 14-22.*
17. *Xiao JB, Högger P. Advances in the pharmacokinetics of natural bioactive polyphenols. Curr Drug Metab 2014; 15(1): 23-29.*
18. *Necyk C, Zubach-Cassano L. Natural Health Products and Diabetes: A Practical Review. Can J Diabetes 2017; 41(6): 642-647.*
19. *Rasines-Perea Z, Teissedre PL. Grape Polyphenols' effects in human cardiovascular diseases and diabetes. Molecules 2017; 22(1): E68.*