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RESEARCH ARTICLE

# Antidiabetic Properties and Mechanism of Action of *Orthosiphon stamineus* Benth Bioactive Sub-fraction in Streptozotocin-induced Diabetic Rats

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**KEYWORDS**
anti-hyperglycemic;
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*Orthosiphon stamineus*

**Abstract**
*Orthosiphon stamineus* is a popular folk medicine widely used to treat many diseases including diabetes. Previous studies have shown that the sub-fraction of chloroform extract was able to inhibit the rise of blood glucose levels in a glucose tolerance test. This study was carried out to evaluate the chronic effect and possible mechanism of action of the bioactive chloroform sub-fraction of *O. stamineus* using streptozotocin-induced diabetic rats and *in vitro* methods. Administration of the chloroform extract sub-fraction 2 ($C_f$2-b) at a dose of 1 g/kg twice daily on diabetic rats for 14 days showed a significant lowering ($p < 0.05$) of the final blood glucose level compared to the pretreatment level. However, there were no significant differences in the plasma insulin levels post-treatment compared to the pretreatment levels for all doses of $C_f$2-b. Conversely, $C_f$2-b at a concentration of 2 mg/mL significantly increased ($p < 0.001$) the glucose uptake by the rat diaphragm muscle. The increase in glucose uptake was also shown when the muscle was incubated in a solution containing 1 IU/mL of insulin or 1 mg/mL of metformin. Furthermore, the effect of this sub-fraction on glucose absorption in the everted rat jejunum showed that $C_f$2-b at concentrations of 0.5 mg/mL, 1 mg/mL and, 2 mg/mL significantly reduced the glucose absorption of the jejunum ($p < 0.05$–$0.001$). Similarly, the absorption of glucose was also inhibited by 1 mg/mL and 2 mg/mL of metformin ($p < 0.001$). These results suggest that the effect of $C_f$2-b may be due to extra-pancreatic mechanisms. There was no evidence that the plant extract stimulated the release of insulin in order to lower the blood glucose level.

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Original Article

# *Orthosiphon stamineus* as a potential antidiabetic drug in maternal hyperglycemia in streptozotocin-induced diabetic rats

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ABSTRACT

*Background:* Maternal hyperglycemia is associated with increased risk of adverse outcomes for both mother and offspring. Insulin is the standard treatment of hyperglycemia with the aim to reduce risks of complications, however, due to several restrictions, the search for more effective drugs from traditional medicinal plants continues.

*Methods:* The antidiabetic effects of *Orthosiphon stamineus* (*O.stamineus*) in non-pregnant and pregnant streptozotocin-induced Sprague Dawley rats were identified. The effect of different concentrations of *O. stamineouson* insulin level using isolated pancreatic islets in response to low and high concentrations of glucose was identified. Oral glucose tolerance test was performed in both pregnant and non-pregnant rats prior to and after treatment with *O. stamineus* (0.1 g/100 g of body weight). *O. stamineus* was given orally daily for 2 weeks in non-pregnant and 10 days in pregnant rats.

*Results:* Oral glucose tolerance test indicated that treatment with *O. stamineus* in non-pregnant and pregnant rats significantly reduced blood glucose level and stimulated glucose-induced insulin secretion. No mortality was recorded throughout the study and no signs of toxicity during the experimental period including in both mother and foetus. For plasma analysis, the interactions of peptides such as GLP-1 and ghrelin level might contribute to the glucose lowering effect by *O. stamineus* via stimulation of insulin. The incubation of islets showed that *O. stamineus*significantly stimulated insulin release in response to high glucose.

*Conclusion:* *O. stamineus* could be a potential source of a specific oral hypoglycaemic agent to treat glucose intolerance in pregnancy.



## 1. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance in pregnancy which includes women with undiagnosed pre-existing diabetes and with first-onset hyperglycemia without history of pre-existing diabetes mellitus.[1,2] Plasma glucose level for GDM when diagnosed, is either above 5.6 mmol/L (fasting) or 7.8 mmol/L (after 2-h), as defined by National Institute for Health and Care Excellence (NICE) Guidelines.[3] Hyperglycemia in pregnancy leads to increased risk of various adverse maternal and infant outcomes such as caesarean delivery, preeclampsia, shoulder dystocia, macrosomia, neonatal hypoglycemia and perinatal death.[4,5]

Insulin has been used for a long time in diabetes management when dietary and lifestyle modifications inadequately achieve persistent euglycaemia. However, administration of insulin requires multiple daily injections causing discomfort to patients. In addition to insulin, for the past few years, there has been increasing research conducted including the observational, randomized controlled trials studies and various types of diabetic animal models to identify if the use of certain oral hypoglycaemic agents such as metformin and glyburide may be suitable in pregnancy. Oral agents have been increasingly viewed to be a potential substitute to insulin

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RESEARCH ARTICLE

# Does *Orthosiphon stamineus* Benth. enhance GLUT4 translocation in the skeletal muscle of induced type II diabetic rats?

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## ABSTRACT

**Background:** GLUT4 acts as the insulin-responsive glucose transporter, thus promoting the uptake of glucose from circulation into muscle and adipose tissue. **Aims and Objectives:** The present study was conducted to determine the effect *Orthosiphon stamineus* Benth. extract on GLUT4 translocation in the skeletal muscle of type II diabetic rats using immunofluorescent technique. **Materials and Methods:** Male Wistar rats aged 10 weeks (180–220 g) were divided into four groups: Normal control group (vehicle receiving group), positive control group (diabetic rats treated with 5 mg/kg metformin), negative control group (diabetic rats not receiving any treatment), and treated group (diabetic rats treated with 1 g/kg *O. stamineus* Benth. aqueous leaf extract). The rats were treated daily for 14 days where the fasting blood glucose level was measured daily; meanwhile, serum insulin was measured before (after injected with STZ-NAD) and after they get treated (at the end of 14 days). At the end of the treatment, the rats were sacrificed and soleus muscles were dissected, fixed in 10% buffered formalin, and processed for immunofluorescence technique. **Results:** The findings showed significantly reduced ($P < 0.01$) mean fasting glucose concentration in positive control and treated groups. Relative pancreas weight and serum plasma concentration were similar in all groups. In the diabetic rats treated with *O. stamineus* Benth., improvement in the translocation activity of GLUT4 was observed. **Conclusion:** Our findings suggest that *O. stamineus* Benth. has the potential in the treatment of Type II diabetes mellitus though the improvement of GLUT4 translocation activity.

**KEYWORDS:** Diabetes Mellitus; GLUT 4; Immunofluorescent Technique; Metformin; *Orthosiphon stamineus* Benth

## INTRODUCTION

Insulin-stimulated glucose transport into skeletal muscle is important in glucose homeostasis. Deviations in insulin-mediated glucose utilization are responsible for Type II diabetes mellitus which can be due to the development of insulin resistance or the impairment of insulin secretion from the beta cells in the Islet of Langerhans.[1] The development of insulin resistance has been linked to the impairment of glucose transport across the peripheral tissues, in particular, the skeletal muscle.[2]

GLUT is a glucose transporter protein which is expressed in insulin-sensitive cells such as adipose tissue, skeletal muscle cells, and cardiomyocytes. GLUT isoform resides mainly in the intracellular membrane compartment under low insulin condition and is translocated to the plasma membrane on stimulation by insulin.[3] To date, 14 glucose transporter's isoforms have been identified and one or more of these GLUT isoforms are expressed in almost every type of human cell, with more expression of GLUT4 identified in the human skeletal muscle.[4-6]

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# α-Glucosidase Inhibitory Activity and Formulation of Solid Lipid Nanoparticle Containing Ethanol 70% Standardized Extract of Kumis Kucing Leaves (*Orthosiphon stamineus* Benth.)

# (Aktivitas Penghambatan α-Glukosidase dan Formulasi Solid Lipid Nanoparticle mengandung Ekstrak Etanol 70% Daun Kumis Kucing Terstandar (*Orthosiphon stamineus* Benth.))

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**Abstract:** Kumis kucing leaves (Orthosiphon stamineus Benth.) are one type of plants that is able to inhibit the activity of α-glucosidase enzyme. This study aims to formulate Solid Lipid Nanoparticle from ethanol 70% standardized extract of kumis kucing leaves and which give α-glucosidase enzyme inhibitory activity. The result of SLN characteristic for particle size of formula I, II and III shows the result of 51,60 nm; 12.81 nm; 11.87 nm and zeta potential of formula I, II and III show the results -27.67; -10,7; -15.0, respectively. The results of the α-glucosidase enzyme inhibitory activity of the standardized extract, SLN formula I, II and III respectively showed IC50 of 132.9 ppm; 130.3 ppm; 131.4 ppm; 132.2 ppm and Acarbose as comparison showed IC50 of 50.0 ppm. Data processing using t-test statistically at α = 0,05 showed that extract and SLN of formula I had significant difference, thus the best SLN formula was formula I with concentration of tween 80 and propylenglycol of 6% and 10%.

**Keywords:** kumis kucing leaves, standardized extract, nanoparticle, solid lipid nanoparticle.

**Abstrak:** Daun kumis kucing (*Orthosiphon stamineus* Benth.) adalah salah satu jenis tumbuhan yang mampu menghambat aktivitas enzim *α-glucosidase*. Penelitian ini bertujuan untuk memformulasi *Solid Lipid Nanoparticle* dari ekstrak etanol 70% daun kumis kucing terstandar dan yang memberikan aktivitas penghambatan enzim *α-glucosidase*. Hasil karakteristik SLN untuk ukuran partikel formula I, II dan III menunjukkan hasil 51,60 nm; 12,81 nm; 11,87 nm dan zeta potensial formula I, II dan III menunjukkan hasil -27,67; -10,7; -15,0. Hasil aktivitas penghambatan enzim *α-glucosidase* dari ekstrak terstandar, SLN formula I, II dan III masing-masing menunjukkan $IC_{50}$ adalah 132,9 ppm;130,3 ppm; 131,4 ppm; 132,2 ppm dan Akarbosa sebagai pembanding menunjukkan $IC_{50}$ yaitu 50,0 ppm. Pengolahan data menggunakan *t-test* secara statistik pada α=0,05 menunjukkan bahwa ekstrak dan SLN formula I memiliki perbedaan yang signifikan, sehingga formula SLN terbaik adalah formula I dengan konsentrasi tween 80 dan propilen glikol adalah 6% dan 10%.

**Kata kunci**: daun kumis kucing, ekstrak terstandar, nanopartikel, *solid lipid nanoparticle*.

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# *Orthosiphon stamineus* sebagai Terapi Herbal Diabetes Melitus

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**Abstrak**

Diabetes melitus (DM) merupakan penyakit metabolik yang diklasifikasikan menjadi empat tipe, yaitu DM tipe I, tipe II, DM karena kehamilan, dan DM tipe sekunder. Diabetes melitus menjadi masalah kesehatan dunia dengan peningkatan angka kejadian yang konstan hingga diperkirakan mencapai 366 juta pada 2030 dan kasus terbanyak adalah DM tipe 2. Penurunan aktivitas fisik, peningkatan obesitas, stres, perubahan pola makan, dan gaya hidup yang tidak sehat merupakan faktor yang memicu peningkatan prevalensi DM. Kontrol ketat terhadap glukosa darah dan pengobatan farmakologi dengan insulin atau obat hipoglikemik oral (OHO) dibutuhkan sebagai pengobatan DM. Pengobatan farmakologi tersebut tentunya memiliki kelemahan, seperti biaya yang tinggi, berbagai efek samping, serta kegagalan terapi. Berkaitan dengan hal ini, WHO pada tahun 1980 merekomendasikan penggunaan tanaman sebagai bahan alami dalam pencegahan dan penyembuhan penyakit DM terutama untuk meminimalisir biaya pengobatan yang tinggi. Salah satu tanaman yang memiliki khasiat antidiabetik adalah *Orthosiphon stamineus* atau sering disebut dengan kumis kucing. Artikel ini bertujuan untuk membahas lebih lanjut manfaat tanaman *O. stamineus* sebagai terapi herbal untuk diabetes melitus berdasarkan penelitian-penelitian sebelumnya. *Orthosiphon stamineus* mengandung berbagai senyawa yang memiliki khasiat menurunkan kadar glukosa darah. Senyawa-senyawa tersebut antara lain adalah orthosiphon glukosa, minyak atsiri, saponin, polifenol, flavonoid, sapofonin, garam kalium, dan mionositol. Berbagai penelitian yang telah dilakukan menunjukkan bahwa jumlah pemberian ekstrak *O. stamineus* sebanyak 800-1000 g/kg secara signifikan dapat menurunkan kadar glukosa darah dan respon ini memiliki efektivitas yang mendekati efek terapi glibenclamide (5 mg/kg). Selain itu, pemberian ekstrak *O. stamineus* juga ternyata dapat meningkatkan kadar HDL dan menurunkan kadar trigliserid.

**Kata Kunci :** diabetes melitus, *Orthosiphon stamineus*, terapi herbal

# *Orthosiphon stamineus* as Herbal Medicine of Diabetes Mellitus

**Abstract**

Diabetes mellitus (DM) is a metabolic disorder that classified into 4 groups, that are type 1 DM, type 2 DM, gestasional diabetes, and secondary diabetes. Diabetes mellitus is global health problem with constant prevalence increasing so that the number of people with diabetes is expected to rise to 366 million in 2030 and most of them are type 2 diabetes. Decreased of physical activities, increased obesity, stress, changes in food consumption, and unhealthy lifestyle are the factors that increase prevalence of diabetes. Tight control of blood glucose and pharmacological treatment by insulin and oral hypoglikemic agent are needed as diabetes therapy. The pharmacological treatment of DM can not be separated from the high cost, side effects, and treatment failure. Because of that, WHO in 1980 recommended the use of plants as natural ingredients to prevent and cure of DM, especially to minimize the high cost of treatment. A plant that is often used as antidiabetic agent is *Orthosiphon Stamineus* or often called kumis kucing. This article is written to review about the benefit of *O. stamineus* as antidiabetic agent according to the previous researches. *Orthosiphon stamineus* contains a lot of substances that act in decreasing blood glucose. They are glucose orthosiphon, atsiri oil, saponin, polifenol, flavonoid, sapofonin, potassium salt, and myonositol. Several studies have been investigated that 800-1000 mg/kg of extract *O. stamineus* most effective in decreasing plasma glucose concentrations and the response is closed to the effectivity of glibenclamide (5 mg/kg). Furthermore, the extract also increase the plasma HDL-cholesterol concentration and decrease plasma triglyceride concentration.

**Kata Kunci :** diabetes mellitus, herbal medicine, *Orthosiphon stamineus*,

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## Pendahuluan

Diabetes melitus (DM) merupakan kelainan metabolik yang menyerang metabolisme karbohidrat, protein, dan lemak.[1] DM dapat diklasifikasikan menjadi empat kelompok, yaitu DM tipe I, tipe II, DM karena kehamilan, dan DM tipe sekunder akibat kerusakan pankreas.[2]

Pada DM tipe 1 terjadi kerusakan pankreas berat, produksi insulin tidak ada atau minimal, sehingga mutlak memerlukan insulin dari luar tubuh. DM tipe 1 dapat timbul pada umur muda (anak-anak atau remaja).[2]

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RESEARCH ARTICLE Open Access

# Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from *Orthosiphon stamineus* Benth as anti-diabetic mechanism

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**Abstract**

**Background:** In the present study, we tested a 50% ethanolic extract of *Orthosiphon stamineus* plants and its isolated bioactive compound with respect to their α-glucosidase and α-amylase inhibitory activities.

**Methods:** Bioactive flavonoid sinensetin was isolated from 50% ethanolic extract of *Orthosiphon stamineus*. The structure of this pure compound was determined on the NMR data and the α-glucosidase and α-amylase inhibitory activities of isolated sinensetin and 50% ethanolic extract of *Orthosiphon stamineus* were evaluated.

**Results:** *In vitro* studies of a 50% ethanolic extract of *O. stamineus* and the isolated sinensetin compound showed inhibitory activity on α-glucosidase ($IC_{50}$: 4.63 and 0.66 mg/ml, respectively) and α-amylase ($IC_{50}$: 36.70 mg/ml and 1.13 mg/ml, respectively). Inhibition of these enzymes provides a strong biochemical basis for the management of type 2 diabetes via the control of glucose absorption.

**Conclusion:** Alpha-glucosidase and α-amylase inhibition could the mechanisms through which the 50% ethanolic extract of *O. stamineus* and sinensetin exert their antidiabetic activity, indicating that it could have potential use in the management of non-insulin-dependent diabetes.

## Background

The number of diabetic patients is rapidly rising in most parts of the world, especially in developing countries such as Thailand, India and Indonesia. In general, the control of blood glucose concentrations near the normal range is mainly based on the use of oral hypoglycaemic/antihyperglycaemic agents and insulin. However, all of these treatments have limited efficacy and are associated with undesirable side effects [1-3], leading to increasing interest in the use of medicinal plants for the alternative management of type 2 diabetes mellitus. The control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and for reducing chronic vascular complications [4]. A sudden increase in blood glucose levels, which causes hyperglycaemia in type 2 diabetes patients, occurs due to the hydrolysis of starch by pancreatic α-amylase and the uptake of glucose by intestinal α-glucosidases [5]. An effective strategy for type 2 diabetes management is the strong inhibition of intestinal α-glucosidases and the mild inhibition of pancreatic α-amylase [6]. One therapeutic approach for reducing postprandial hyperglycaemia in patients with diabetes mellitus is preventing the absorption of carbohydrates after food uptake. Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the bloodstream. Complex starches, oligosaccharides and disaccharides must be broken down into individual monosaccharides before they are absorbed in the duodenum and upper jejunum. This digestion is facilitated by enteric enzymes, including pancreatic α-amylase and α-glucosidases attached to the brush border of intestinal cells.

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# PERBANDINGAN EFEKTIFITAS EKSTRAK ETANOL 96% AKAR DAN DAUN KUMIS KUCING (*Orthosiphon stamineus*) TERHADAP PENURUNAN GLUKOSA DARAH MENCIT (*Mus musculus*)

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**Abstract: Comparison Effectiveness of Extract Ethanol 96% Root and Leaves Kumis Kucing (Orthosiphon Stamineus) Against Reduction of Blood Glucose Mice (Mus Musculus).** *Orthosiphon stamineus* is known as kumis kucing, a traditional plant which used widely in many diseases, including diabetes mellitus. Riset Kesehatan Dasar (Riskesdas) 2018 shown the prevalence of diabetes mellitus is getting higher from 6,9 % becoming 8,5 %. Objective: To compared the effectiveness of 96% ethanol extract between leaves and roots of kumis kucing (*Orthosiphon stamineus*) to decreased mice's (*Mus musculus*) blood glucose levels. Method: This was an experimental study, 25 mice were induced alloxan in I.P, leaf extract and root of kumis kucing were administered at 35 mg and 50 mg/ 20 gBB /day doses for 7 days. Result: The result showed that the extract of the leaves of kumis kucing dose 35 mg and 50 mg / 20 gBB / day decreased the blood glucose level 35.45 % and 41.61 %, whereas the root extract of kumis kucing dose 35 mg and 50 mg/20 gBB/day as much as 25.5 % and 29, 19 %. Paired t test obtained p value = 0.000, it's mean there is a significant decreasing in blood glucose levels. Conclusion: 96% ethanol extract of kumis kucing's leaves with dosage 50 mg/20 gBB/ day reduce 41,6 % of the blood glucose level.

**Keywords:** Leaf and root extract of kumis kucing, blood glucose levels, diabetes mellitus.

**Abstrak: Perbandingan Efektifitas Ekstrak Etanol 96% Akar Dan Daun Kumis Kucing (*Orthosiphon stamineus*) Terhadap Penurunan Glukosa Darah Mencit (*Mus musculus*)** *Orthosiphon stamineus* yang dikenal dengan nama kumis kucing adalah tanaman herbal yang banyak digunakan dalam berbagai penyakit, termasuk diabetes melitus. Tanaman ini sangat mudah dijumpai di Indonesia. Hasil Riset Kesehatan dasar (Riskesdas) 2018 diperoleh data prevalensi diabetes melitus meningkat dari 6,9 % menjadi 8,5 %. Membandingkan efektivitas ekstrak etanol 96% daun dengan akar kumis kucing (*Orthosiphon stamineus*) terhadap penurunan kadar glukosa darah mencit (*Mus musculus*). Jenis penelitian ini adalah eksperimental, 25 ekor mencit diinduksikan aloksan secara I.P, ekstrak daun dan akar kumis kucing masing-masing diberikan dengan dosis 35 mg dan 50 mg/ 20 gBB /hari secara oral selama 7 hari. Hasil: Ekstrak daun kumis kucing dosis 35 mg dan 50 mg/20 gBB/ hari menurunkan kadar glukosa darah mencit 35,45% dan 41,61%, sedangkan eskstrak akar kumis kucing dosis 35 mg dan 50 mg/20 gBB/ hari sebanyak 25,5% dan 29,19%. Uji t berpasangan didapatkan nilai p = 0.000, artinya terdapat perbedaan penurunan kadar glukosa darah mencit yang signifikan. Kesimpulannya yaitu Ekstrak etanol 96% dari daun kumis kucing dosis 50 mg/20 gBB/ hari memiliki efek antidiabetik yang lebih tinggi daripada ekstrak akar kumis kucing.

**Kata Kunci**: Ekstrak daun dan akar kumis kucing, kadar glukosa darah mencit, diabetes melitus.

# STUDI *MOLECULAR DOCKING* SENYAWA FLAVONOID HERBA KUMIS KUCING (*Orthosiphon stamineus* B.) PADA RESEPTOR α-GLUKOSIDASE SEBAGAI ANTIDIABETES TIPE 2

## *MOLECULAR DOCKING STUDY FLAVONOID COMPOUNDS FROM KUMIS KUCING (Orthosiphon stamineus B.) IN α-GLUKOSIDASE RECEPTOR AS ANTIDIABETIC TYPE 2*

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**ABSTRAK**

Diabetes Mellitus (DM) adalah suatu penyakit dimana kadar gula (glukosa) di dalam darah tinggi karena tubuh tidak dapat melepaskan atau menggunakan insulin secara efektif. Kumis kucing (*Orthosiphon stamineus* B.) mempunyai khasiat sebagai antidiabetes. Penelitian ini bertujuan untuk mengetahui apakah senyawa turunan flavonoid pada herba kumis kucing berpotensi sebagai obat antidiabetes-tipe 2 melalui mekanisme penghambatan α-glucosidase. Jenis penelitian ini adalah *in silico* menggunakan metode *molecular docking*. Proses *docking* dilakukan menggunakan *Autodock Vina*. Simulasi *molecular docking* dilakukan untuk menguji potensi senyawa flavonoid yang terkandung dalam kumis kucing sebagai kandidat obat antidiabetes sebagai inhibitor alami enzim α-glukosidase melalui parameter nilai energi bebas Gibbs, konstanta inhibisi, ikatan hidrogen dan residu asam amino. Hasil simulasi menunjukan senyawa flavonoid yang memiliki nilai energi bebas Gibbs terbaik yaitu 5,6,7,3'-tetrametoksi-4'hidroksi-8-C-prenylflavon sebesar -8.2 Kkal/mol sedangkan nilai energi bebas Gibbs acarbose adalah -8.7 Kkal/mol. Kesimpulan penelitian ini menunjukan bahwa senyawa flavonoid pada kumis kucing belum berpotensi untuk dijadikan sebagai kandidat obat antidiabetes-tipe 2 dalam menggantikan akarbose melalui mekanisme penghambatan α-glucosidase.

**Kata Kunci:** Diabetes Tipe 2, *Orthosiphon stamineus* B, α-Glucosidase, *Moleculer Docking*

***ABSTRACT***

*Diabetes Mellitus (DM) is a disease in which the level of sugar (glucose) in the blood is high because the body cannot release or use insulin effectively. Kumis kucing (Orthosiphon stamineus B.) has antidiabetic properties. This study aims to determine whether the flavonoid derivatives in kumis kucing have the potential as an antidiabetic drug-type 2 through the α-glucosidase inhibition mechanism. This type of research is in silico using the molecular docking method. Docking process was performed using Autodock Vina. Molecular docking simulations were carried out to test the potential of flavonoid compounds contained in cat whiskers as antidiabetic drug candidates as natural inhibitors of the α-glucosidase enzyme through the parameters of Gibbs free energy values, inhibition constants, hydrogen bonds and amino acid residues. The simulation results show that the flavonoid compound that has the best Gibbs free energy value is 5,6,7,3'-tetramethoxy-4'hydroxy-8-C-prenylflavon of -8.2 Kcal / mol while the Gibbs acarbose free energy value is -8.7 Kcal / mole. The conclusion of this study shows that the flavonoid compounds in cat whiskers are not yet potential to be used as candidates for antidiabetic drug-type 2 in replacing acarbose through the α-glucosidase inhibition mechanism.*

***Keywords:*** *Diabetes Type 2, Orthosiphon stamineus B, α-Glucosidase, Molecular Docking*

## PENDAHULUAN

Diabetes Mellitus atau sering disebut dengan kencing manis adalah suatu penyakit kronik yang terjadi ketika tubuh tidak dapat memproduksi cukup insulin atau tidak dapat menggunakan insulin (resistensi insulin), dan di diagnosa melalui pengamatan kadar glukosa di dalam darah (IDF, 2015). Salah satu obat antidiabetes oral adalah golongan penghambat α-glukosidase, yaitu acarbose (Soegondo, 2011). α-glukosidase merupakan enzim yang berperan dalam proses metabolisme karbohidrat terletak dibagian tepi permukaan sel usus halus, dan proses pembentukan

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# Effect of ultraviolet and ultrasonic on potential antidiabetic activity of in vitro shoot cultures of *Orthosiphon aristatus*

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**Abstract**. *Orthosiphon aristatus Boldingh*., a native tropical plant from Indonesia, is a medicinal herb that has been reported to possess an antidiabetic potential. However, study on this functional activity is limited especially on in vitro plant culture of *Orthosiphon aristatus*. The present study sought to investigate the effect of ultraviolet (UV) and ultrasonic (US) on this potential for *in vitro* shoot cultures of *Orthosiphon aristatus* (ISCOA). Methanol (70% v/v) extracts of ISCOA had the ability to inhibit alpha-amylase and alpha-glucosidase enzymes, dependent on the concentration of the extract. Among all treatment, highest inhibition of α-amylase and α-glucosidase activities were observed in combined treatment between UV for 60 minutes and US for 6 minutes. This was showed by the highest reduction by 0.4 point of IC50 inhibitory α-amylase and alpha-glucosidase activities in day 3 and in day 1 after exposure UV and US, respectively. The combination of UV and US has been shown to be effective in improving potential anti-diabetic properties of *in vitro* shoot cultures of Orthosiphon aristatus in which correlated with the increasing level of secondary metabolites.

## 1. Introduction

*Orthosiphon aristatus* is a native tropic-medicinal plant from Southeast Asia. It belongs to Lamiaceae family. This plant has been widely used as a traditional/natural alternative medicine in Indonesia, Malaysia and Thailand for the treatment of various diseases; for instance, gout, diabetes mellitus, hypertension, rheumatism, tonsillitis and menstrual disorder, and especially those affecting the urinary tract, that is for treating kidney ailments and bladder related diseases (18).

Many studies have indicated that *O. aristatus* exhibits several secondary metabolic active constituents. These compounds contribute to the plant's potential medicinal properties. However, the pharmacologically effects of *O. aristatus* have been recommended mainly due to its phenolics compounds (16) which are the most dominant constituent in the leaf. These phenolic compounds had been reported to be effective in reducing oxidative stress by inhibiting the formation of lipid peroxidation products in biological systems (17). One of the most important phenolic compounds in O.

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*Article*

# Antihyperglycemic Effect of *Orthosiphon Stamineus* Benth Leaves Extract and Its Bioassay-Guided Fractions

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**Abstract:** Preliminary investigations were carried out to evaluate the antidiabetic effects of the leaves of *O. stamineus* extracted serially with solvents of increasing polarity (petroleum ether, chloroform, methanol and water); bioassay-guided purification of plant extracts using the subcutaneous glucose tolerance test (SbGTT) was also carried out. Only the chloroform extract, given at 1 g/kg body weight (b.w.), significantly reduced ($P < 0.05$) the blood glucose level of rats loaded subcutaneously with 150 mg/kg (b.w.) glucose. The active chloroform extract of *O. stamineus* was separated into five fractions using a dry flash column chromatography method. Out of the five fractions tested, only chloroform fraction 2 (C*f*2), at the dose of 1 g/kg (b.w.) significantly inhibited ($P < 0.05$) blood glucose levels in SbGTT. Active C*f*2 was split into two sub-fractions C*f*2-A and C*f*2-B, using a dry flash column chromatography method. The activities C*f*2-A and C*f*2-B were investigated using SbGTT, and the active sub-fraction was then further studied for anti-diabetic effects in a streptozotocin-induced diabetic rat model. The results clearly indicate that C*f*2-B fraction exhibited a blood glucose lowering effect in fasted treated normal rats after glucose-loading of 150 mg/kg (b.w.). In the acute streptozotocin-induced diabetic rat model, C*f*2-B did not exhibit a hypoglycemic effect on blood glucose levels up to 7 hours after treatment. Thus, it appears that C*f*2-B functions similarly to metformin, which has no

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# Genotype selection for phytochemical content and pharmacological activities in ethanol extracts of fifteen types of *Orthosiphon aristatus* (Blume) Miq. leaves using chemometric analysis

Irmanida Batubara[1,2], Komariah Komariah[3], Apong Sandrawati[4] & Waras Nurcholis[1,5]

***Orthosiphon aristatus*** **(Blume) Miq. of the *Lamiaceae* family, called as kumis kucing in Indonesia, is a valuable medicinal plant for their pharmacological properties. The present study comprised of fifteen genotypes of *O. aristatus* was undertaken to evaluate the genotypes based on phytochemical content and pharmacological activities of leaves ethanol extract. Chemometric analysis (correlation and principal component analysis) was also used to investigate the genetic variability based on phytochemical content and pharmacological activities of *O. aristatus* genotypes. Results of phytochemical characterization showed that total phenolic ranged from 1.48 to 36.08 (maximum in A15) mg GAE/g DW, total flavonoid ranged from 0.10 to 3.07 (maximum in A15) mg QE/g DW, sinensetin ranged from 0.36 to 4.02 (maximum in A11) mg/g DW, and rosmarinic acid ranged 0.06 to 7.25 (maximum in A7) mg/g DW. Antioxidant activity was tested using DPPH and FRAP assay. Antioxidant results showed that DPPH ranged from 1.68 to 15.55 (maximum in A15) µmol TE/g DW and FRAP ranged from 0.07 to 1.60 (maximum in A1 and A7) µmol TE/g DW. The genotype A8 showed the highest cytotoxic activities against HeLa (66.25%) and MCF-7 (61.79%) cell lines. Maximum α-glucosidase inhibitory activity was recorded in genotype A2 with the value of 62.84%. The genotypes A1, A2, A7, A11, and A15 were identified as superior based on their phytochemicals content and pharmacological activities coupled with chemometric analysis. This finding is important for breeding studies and also the pharmaceutical perspective of *O. aristatus*.**

**Abbreviations**

| | |
|---|---|
| DPPH | 2,2-Diphenyl picrylhydrazyl |
| FRAP | Ferric reducing antioxidant power |
| TE | Trolox equivalent |
| DW | Dry weight |
| GAE | Gallic acid equivalent |
| QE | Quercetin equivalent |
| TPC | Total phenolic content |
| TFC | Total flavonoid content |

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Research Article

# Chemical Compound Identification of Two Varieties Cat of Whiskers (*Orthosiphon aristatus* Blume Miq) from *In Vitro* Culture

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**Abstract** | The cat's whiskers plant (*Orthosiphon aristatus* Blume Miq) is empirically used in Indonesia, Malaysia, Australia, and Southern Asia as an antibacterial, antidiabetic, antihypertensive anti-inflammatory. The country of Indonesia has three varieties of *O. aristatus*, namely purple, white-purple and white. The purple and white-purple population has started to decline. Efforts are needed to maintain the two varieties' population, one of which is plant tissue culture. It is hoped that *in vitro* cultured plants contain the same secondary metabolites as the wild type. This study aims to identify the chemical content of two varieties of *O. aristatus* resulting from *in vitro* culture. The five-month-old variety of *O. aristatus* purple and white purple and wild type was extracted using two solvents with different polarity levels, namely ethanol and ethyl acetate. The concentrated extract was identified for its chemical content using gradient system HPLC 0.1% formic acid: acetonitrile. The qualitative analysis results showed that the two extracts of *O. aristatus* resulted from *in vitro* culture containing rosmarinic acid and sinensetin. When compared to the wild type, the chromatogram had a larger area than the wild type. This study provides new information regarding the secondary metabolite content of two *O. aristatus* from *in vitro* culture.

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**DOI** | https://dx.doi.org/10.17582/journal.sja/2021/37.4.1355.1363
**Keywords** | *O. aristatus*, *In-vitro* culture, Wild type, Secondary metabolite, Qualitative analysis

## Introduction

Since 2019, there was a pandemic due to COVID-19, the search for sources or medicinal plants that can act as antiviral and immunomodulators. One of the plants that have the potential for this activity is *O. aristatus*. Several studies have reported the potential of the extract and some chemical properties of the *O. aristatus* plant as an antiviral (Faramayuda *et al.*, 2021b). Water extract of leaves, flowers, and all the plants in addition to the root of the *O. aristatus* (0.39 mg/ mL) had high antiviral activity observed after normal cells (Vero cells) were inoculated with herpes simplex virus type 1 (HSV-1) (post-treatment) with a 100% reduction in HSV-1 plaque. In the pre-treatment test, leaf water extract, flowers, and all parts of plants other than roots showed HSV-1 plaque reduction activity was 79%, 84%, and 97% using the same concentration (Ripim *et al.*, 2018). The chemical content in *O. aristatus* that has the potential to be antiherpetic is caffeic acid, eugenol, N-transferulolyl tyramine, limonene, β-caryophyllene, beta-pinene, p-cymene (Ikeda *et al.*, 2011; Bourne *et al.*, 1999; Benencia and Courreges,

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RESEARCH ARTICLE

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# Comprehensive chemical and metabolic profiling of anti-hyperglycemic active fraction from Clerodendranthi Spicati Herba

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Extensive pharmacological research has demonstrated that Clerodendranthi Spicati Herba has an obvious anti-hyperglycemic effect via α-glucosidase inhibitory activity. However, the anti-hyperglycemic active fraction and its metabolic behavior in vivo have not been elaborated clearly. In this study, ultra-high-performance liquid chromatography coupled to quadrupole time of flight tandem mass spectrometry with data filtering strategy, including mass defect screening, diagnostic product ions and neutral loss identification, was established for chemical and metabolic profiling of anti-hyperglycemic active fraction from Clerodendranthi Spicati Herba. A total of 28 methoxylated flavonoids and 61 diterpenoids were rapidly identified. Four main known methoxylated flavonoids were purified and unambiguously identified by nuclear magnetic resonance analysis. Thirty-one absorbed diterpenoids, 12 absorbed methoxylated flavonoids, and 56 methoxylated flavonoids metabolites were identified in rat plasma, urine, bile, and feces after oral administration of anti-hyperglycemic active fraction. The methoxylated flavonoids were predominantly metabolized by demethylation, sulfation, and glucuronidation. Glucuronidation metabolites found in bile and urine after demethylation were dominant metabolites. Diterpenoids were absorbed into the blood mainly in the form of prototypes and excreted through bile and urine. These results indicated that methoxylated flavonoids and diterpenoids were responsible for α-glucosidase inhibitory activity, which might provide novel drug candidates for the management of diabetes mellitus.

**KEYWORDS**
anti-hyperglycemic effect, Clerodendranthi Spicati Herba, data filtering strategy, diterpenoids, methoxylated flavonoids

## 1 | INTRODUCTION

Clerodendranthi Spicati Herba (CSH), the dried aerial part of *Clerodendranthus spicatus* (Thunb.) C.Y. Wu, was traditionally used as an edible and medicinal material in China, Malaysia, Vietnam, Indonesia, and other Southeast Asian countries, which is commonly applied to treat diabetes,

**Article Related Abbreviations:** AUC, area under the curve; CSH, Clerodendranthi Spicati Herba; DAD, diode array detector; DPIs, diagnostic product ions; EEF, ethanol elution fraction; MFs, methoxylated flavonoids; NL, neutral loss; RDA, Retro-Diels-Alder

  

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# Selected herbal extracts improve diabetes associated factors in 3T3-L1 adipocytes

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**Abstract**

Owing to the current worsening situation of the increasing burden of diabetes around the world including Malaysia it is worthwhile to discover non-pharmacological prevention or treatment for it. In order to have a useful explanation of the efficacy of herbs or nutrients for diabetes; it is desirable to know the effect on the balance between the adipogenesis, adipolysis and glucose uptake in the adipose tissues. Therefore, four herbs namely *Orthosiphon stamineus* (Cat whisker) (OS), *Peronema canescens* (Sungkai) (PC), *Momordica charantia* (Bitter gourd/bitter melon) (MC) and Pithecellobium *jiringa* (Jering) (PJ) were screened for their antidiabetic properties in *in vitro* model 3T3-L1 adipocytes. Water extracts of these herbs were prepared and evaluated for their effects on cell proliferation, adipogenesis, adipolysis and glucose uptake in 3T3-L1 preadipocytes cells. The aforementioned extracts promoted cell proliferation at a dose of 0.25mg/ml which showed more than 90% viability after 48 hours of treatment. The result of this study indicates that OS extracts significantly ($P<0.001$) increased adipogenesis whereas PC, MC and PJ extracts were not effective compared to control. The extracts from all four plants caused increased lipolysis compared to control. The Extract from OS and PJ significantly ($P<0.05$) stimulated glucose uptake in the cells whereas PC, MC were not effective. When the glucose consumption was compared to control it was significantly ($P<0.001$) increased for all extracts in the medium. The present study provides some important baseline data on the biochemical aspects of the effect induced by the herbs and suggestive of possessing antidiabetic properties which can be exploited for diabetes prevention and associated metabolic dysfunctions.

Keywords: Herbal extracts, 3T3-L1 adipocytes, adipogenesis, adipolysis, glucose uptake

# Study on the developmental toxicity of a standardized extract of *Orthosiphon stamineus* in rats

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**Abstract:** Infusions of *Orthosiphon stamineus* Benth., Lamiaceae, leaves are widely used in Southeastern Asia to treat different illnesses. Nonetheless, no data is available on the safety of *O. stamineus* for pregnant women and their babies. This study was undertaken to evaluate the developmental toxicity of *O. stamineus* standardized aqueous extract in female Sprague Dawley rats (n=21) at 0, 250, 500, 1000 and 2000 mg/kg/day, by gavage on gestation days 6-20. Clinical signs of maternal toxicity, body weight gain, and food and water consumption were recorded. Caesarean sections were performed on gestation day 21; resorptions and living and dead fetuses were counted. Fetuses were weighed and examined for external abnormalities. Half of the fetuses from each litter were cleared and stained with Alizarin red S for skeleton evaluation. *O. stamineus* standardized aqueous extract did not alter pregnancy body weight gain and food and water consumption and caused no other sign of maternal toxicity. Embryolethality and prenatal growth retardation were not observed either. *O. stamineus* standardized aqueous extract increased a few skeleton variations and a skull bone malformation (hyoid bone absent) in a non-dose dependent manner. Anogenital distance was increased in male and female fetuses exposed to the highest *O. stamineus* standardized aqueous extract dose, an indication that the extract could possibly contain androgenic compounds.

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## Introduction

*Orthosiphon stamineus* Benth., Lamiaceae, or "Java tea" is a medicinal plant traditionally used in Southeastern Asia. It is also known as "MisaiKucing" in Malaysia, "Kumis Kucing" in Indonesia, "Balbas-pusa" and "Kabling-gubat" in Philippines, "Kapen prey" in Cambodia, "Hnwàdméew" in Laos, "YaaNuatMaeo" in Thailand, and "Thé de Java" in French speaking countries (Anon, 2001). In Southeastern Asia people are currently exposed to *O. stamineus* through the consumption of infusions made with its leaves, medicinal potions and phytotherapeutic drugs. In Malaysia, a tea made with *O. stamineus* leaves is used to improve health and to treat a variety of diseases such as kidney disorders, bladder inflammation, gout, diabetes, eruptive fevers, hepatitis, hypertension, syphilis, rheumatism and gonorrhoea (Akowuah et al., 2004; Ameer et al., 2012).

Studies on the pharmacological properties of *O. stamineus* extracts seem to lend support to some of their common uses in folk medicine. Anti-oxidant and anti-inflammatory activities as well as a beneficial effect on hyperglycemia and altered lipid profile in diabetic rats have been reported (Arafat et al., 2008). Diuretic properties of aqueous extracts of *O. stamineus* were demonstrated as well (Adam et al., 2009). Methanol (50%) extracts of *O. stamineus*, on the other hand, were described to have anti-pyretic activity (Yam et al., 2009) and to inhibit the growth of food-borne bacteria (*Vibrio parahaemolyticus*) *in vitro* test systems (Ho et al., 2010).

Methoxylated flavones (sinensetin and eupatorin) and phenolic acids (rosmarinic and caffeic acids) were identified in *O. stamineus* leaf extracts (Muhammad et al., 2011; Ameer et al., 2012). It is of note that rosmarinic acid, a major component of aqueous extracts from *O. stamineus* leaves, has been reported to exhibit antioxidant, immuno-

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FULL ARTICLE

Journal of Food Biochemistry WILEY

# Bioassay-guided isolation of anti-inflammatory diterpenoids with highly oxygenated substituents from kidney tea (*Clerodendranthus spicatus*)

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## Abstract

The whole plant of *Clerodendranthus spicatus* (Thunb.) is one of popular functional food in south of China, named as "kidney tea" and used to ameliorate renal inflammation. In order to verify this potential function and explore the accurate compounds responsible for inflammation, the ethanol extract, fractions, and subfractions of this plant were prepared to evaluate anti-inflammation effect on xylene-induced acute inflammatory mice model, and the results indicated that two subfractions from EtOAc fraction show potential activities. Subsequent bioassay-guided isolation of the bioactive subfractions led to isolation of 25 compounds. Among them, compounds **2**, **4**, **5**, **9**–**11**, **13**, **16**, **17**, and **20**–**22** inhibited the productions of pro-inflammation factors TNF-$\alpha$, IL-1$\beta$, and IL-8 in lipopolysaccharide (LPS) -induced renal epithelia (*HK-2*) cells, respectively. Further anti-inflammation evaluation in vivo indicated that the major bioactive compounds **1**, **2**, **5**–**7**, **17**, **21**, and **22** from *C. spicatus* were even better than aspirin.

## Practical applications

*C. spicatus* as a healthy tea has been available in the Chinese market and as a medicine for various disorders such as nephritis, rheumatism, inflammation, gout, and diabetes. Previous pharmacological investigation of the plant revealed the potential anti-inflammatory activities, but the material basis of anti-inflammatory activity remains to be elucidated. In our study, the anti-inflammatory fractions and compounds were obtained by the bioassay-guide isolation and the results showed that the highly oxygenated diterpenoids were major anti-inflammatory compounds, in which **1**, **2**, **5**–**7**, **17**, **21**, and **22** were even better than aspirin. This information supported kidney tea as a functional food for treatment of renal inflammation reasonably and may add a new dimension to biological activity of this plant in the field of agriculture as a functional food were cultivated.

KEYWORDS

anti-inflammation, *Clerodendranthus spicatus*, diterpenoids, flavonoids, kidney tea

**Abbreviations:** AQ, aqueous; ASP, aspirin; DXM, dexamethasone; EtOAc, ethyl acetate; EtOH, ethanol; IL-1$\beta$, interleukin 1$\beta$; IL-8, interleukin 8; LPS, lipopolysaccharide; Ph, phenyl; TNF-$\alpha$, tumor-necrosis factor.

Wei-Di Chen and Yun-Li Zhao contributed equally to this work.

  

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# Bioactivity-guided separation of potential α-glycosidase inhibitor from clerodendranthus spicatus based on HSCCC coupled with molecular docking

Chunsheng Zhu[1,3], Hongjuan Niu[2,3], Anzheng Nie[1]✉ & Meng Bian[1]✉

***Clerodendranthus Spicatus*** **is a traditional Dais medi-edible plant and it has been proven to have good blood glucose-lowering efficacy. However, the material basis of *Clerodendranthus Spicatus* has not been clarified yet and therefore needs to be determined. In this paper, the effective ingredients of this medicine were purified by high-speed counter-current chromatography. Alongside, their potential hypoglycemic activity was determined by α-glucosidase inhibitory activities in vitro and molecular docking. Finally, five compounds were purified and identified as 2-caffeoyl-L-tartaric acid (1), N-(E)-caffeoyldopamine (2), rosmarinc acid (3), methyl rosmarinate (4), 6,7,8,3′,4′-Pentamethoxyflavone (5). Examination of α-glucosidase inhibitory activity in vitro showed that 2-caffeoyl-L-tartaric acid and rosmarinic acid had a higher inhibitory activity than acarbose. Molecular docking indicated that the affinity energy of the identified compounds ranged from − 7.6 to − 8.6 kcal/mol, a more desirable result than acarbose (− 6.6 kcal/mol). Particularly, rosmarinc acid with the lowest affinity energy of − 8.6 kcal/mol was wrapped with 6 hydrogen bonds. Overall, α-glucosidase inhibitory activities and molecular docking suggested that rosmarinc acid was likely to be a promising hypoglycemic drug.**

Diabetes is a serious chronic metabolic disorder affecting 463 million of individuals worldwide in 2019, and there may be an extra 700 million by 2045[1]. Additionally, numerous epidemiological studies have shown that diabetes is not only closely related to blindness, limb amputations, and renal failure, but also an independent risk factor for peripheral artery disease[2–4]. Diabetes can be categorized into two types: T1D is mainly caused by insufficient insulin secretion, and T2D is abnormal insulin secretion and/or non-insulin-dependent diabetes, complicated by postprandial hyperglycemia[5,6]. Remarkably, according to clinical statistics, only 5–10% of diabetic patients are affected by T1D, while T2D accounts for more than 90% of diabetes cases[7,8].

It is well known that the inhibitors of carbohydrate-related enzymes like α-glycosidase by delaying glucose absorption, including acarbose, miglitol, and voglibose, is one of the most effective controlling methods to overcome postprandial hyperglycemia[8]. However, long-term usage of these drugs exhibits a series of undesired side effects on the digestive system, such as abdominal pain, diarrhoea and nausea[9,10]. Accordingly, screening new α-glycosidase inhibitors to develop the new drugs with fewer side effects has been a research hotspot in recent years.

*Clerodendranthus spicatus (Thunb.)* C. Y. Wu (*C. spicatus*), popularly known as "orthosiphon" or "kidney tea", is a perennial herb of the Labiatae family, which is an endemic specie distributed in southern China, such as Yunnan, Fujian and Guangxi provinces[11,12]. *C. spicatus* is a kind of medi-edible plant medicine, which has been used as an herbal remedy for gout, acute and chronic nephritis in Dai medicine with a history of more than 2000 years in local ethnic groups in Yunnan Province, China[13,14]. In addition, an earlier report has indicated that this plant contains high amount of flavonoids, phenolic acids, and anthraquinones[15]. Moreover, modern pharmacological studies have shown that the extracts of *C. spicatus* exhibit a significant effect on hypoglycemic effect, which can significantly lower the blood glucose levels in the streptozotocin-induced diabetes mouse

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methods and protocols

MDPI

*Protocol*

# Solvation Free Energy Simulation for Rosmarinic Acid Extraction from *Orthosiphon stamineus*

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**Abstract:** This study was aimed to extract rosmarinic acid from *Orthosiphon stamineus* Benth. (*Lamiaceae*) in high yield. The mixture of chloroform–ethyl acetate (70:30) was chosen as the solvent system because rosmarinic acid gave the lowest solvation free energy in that solvent system based on the computational solubility prediction. The crude extract of the plant was fractionated by C18 reversed phase absorbent to recover rosmarinic acid. The content of rosmarinic acid was increased from 4.0% *w/w* to 6.7% *w/w* after fractionation. The radical scavenging activity of rosmarinic acid rich fraction ($IC_{50}$ = 38.3 µg/mL) was higher than the crude extract ($IC_{50}$ = 58.85 µg/mL) based on the DPPH assay. Several phytochemicals were also identified based on the detection of fragment ions of target compounds. Fractions 1 to 3 could be combined to be a rosmarinic acid rich fraction. Simultaneously, the combination of fractions 4 to 6 could obtain a plant fraction rich in rosmarinic acid, sinensetin and eupatorin, whereas fractions 7 to 9 could be combined as a sinensetin rich fraction. The preparation of known phytochemical profile of *O. stamineus* fraction is highly required for value added product formulation and pharmacological studies, particularly for anti-diabetes and kidney related diseases which had previously been reported attributed to this herbal plant. This is the first study using solvation free energy to predict the suitable solvent system for rosmarinic acid extraction from highly complex herbal sample using the technology of solid phase extraction. The use of solvation free energy simulation is convenient and reliable before wet experiments for time and cost saving.

**Keywords:** *Orthosiphon stamineus*; radical scavenging activity; solvation free energy; fragment ions; solid phase extraction

## 1. Introduction

*Orthosiphon stamineus* Benth. is a plant of genus *Orthosiphon* in the family *Lamiaceae*, which is widely used in traditional remedy to improve general health, treatment of kidney diseases, bladder inflammation, gout and diabetes in South East Asia countries [1]. The pharmacological effects of the plant might be due to the presence of several dominant phytochemicals such as terpenoids, polyphenols and sterols [2]. The phytochemicals of *O. stamineus* are mostly from the class of polyphenol (rosmarinic acid, caffeic acid and cichoric acid), flavonoid (sinensetin and eupatorin) and terpenoids (orthosiphol A–Z) [3].

Phytochemicals are also known as plant secondary metabolites, which are important for plant defensive system and possess important biological activity, including antioxidant activity [4]. The phenolic compounds with antioxidant property can scavenge free radicals such as hydroxyl and superoxide anion, as well as chelate metal ions [5]. The natural antioxidants from plants show the potentials in human health protection, as well as food preservatives and additives [5]. Rosmarinic acid

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# Toxicity evaluation of a standardised 50% ethanol extract of *Orthosiphon stamineus*

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ABSTRACT

*Aim of the study:* The present investigation was carried out to evaluate the safety of standardised 50% ethanol extract of *Orthosiphon stamineus* plant by determining its potential toxicity after acute and subchronic administration in rats.

*Materials and methods:* For acute toxicity study, up and down method (limit dose) was adapted. A single dose of 5000 mg/kg of the standardised 50% ethanol extract of *O. stamineus* was given orally to 5 healthy Sprague-Dawley (SD) female adult rats. The rats were observed for mortality and clinical signs for 3 h and then periodically for 14 days. While in the subchronic toxicity study, the extract was administered orally at doses of 1250, 2500 and 5000 mg/kg per day for 28 days to female and male SD rats, respectively. The animals were sacrificed, followed by examination of their organs and blood serum.

*Results:* In the acute toxicity study, standardised 50% ethanol extract of *O. stamineus* at a dose of 5000 mg/kg caused neither visible signs of toxicity nor mortality. All five rats survived until the end of observation period. While in subchronic toxicity, administration of the standardised 50% ethanol extract of *O. stamineus* at 1250, 2500, and 5000 mg/kg for 28 days did not produce any mortality and there were no significant differences in the general condition, growth, organ weights, heamatological parameters, clinical chemistry values, or gross and microscopic appearance of the organs from the treatment groups as compared to the control group.

*Conclusions:* Standardised 50% ethanol extract of *O. stamineus* did not cause any death nor did it cause abnormalities in necropsy and histopathology findings. There were no acute or subchronic toxicity observed and this extract could be devoid of any toxic risk. The NOAEL for the standardised 50% ethanol extract of *O. stamineus* is 5000 mg/kg per day for 28 days.



## 1. Introduction

Currently, *Orthosiphon stamineus* Benth [syn: *Orthosiphon aristatus* (B1) Miq., *Orthosiphon grandiflorus* Bold., *Orthosiphon spicatus* (Thumb) Bak.; Lamiaceae] (Suresh et al., 2002) is a popular traditional folk medicine extensively used in Southeast Asia for the treatment of a wide range of diseases including rheumatism, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorders, gonorrhoea, syphilis, renal calculus and gallstones in Indonesia (Bwin and Gwan, 1967); urinary lithiasis, oedema, eruptive fever, influenza, hepatitis and jaundice in Vietnam (Eisai, 1995); and diabetes and urinary tract and renal diseases in Myanmar (WHO, 1987).

Based on its popularity and demonstrated effectiveness, phytochemical studies (Masuda et al., 1992; Sumaryono et al., 1991; Yoshio et al., 1993) and pharmacological studies (Beaux et al., 1999; Doan et al., 1992; Nirdnoy and Muangmanm, 1991) of *O. stamineus* have been conducted since 1930. This plant has been reported to contain highly oxygenated isopimarane-type diterpenes and orthosiphols A–E, along with monoterpenes, triterpenes, saponins, flavonoids, hexoses, organic acids, rosmarinic acid, chromene and myo-inositol (Malterud et al., 1989; Tezuka et al., 2000; Olah et al., 2003). In addition, it has been reported to possess hypoglycaemic and anti-hyperglycaemic activities (Mariam et al., 1996) as an aqueous extract of *O. stamineus* has a hypoglycaemic effect in normal and streptozotocin-induced diabetic rats.

Despite its long use for a variety of conditions, little subchronic or chronic toxicity data are available regarding the safety of repeated exposure to *O. stamineus*. After reviewing the relevant literature, only toxicity data from a study of acute exposure to a single dose and a study of 14 days of repeated exposure were avail-

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**Inhibition of glycosidase by ursolic acid: In vitro, in vivo and in silico study**

**Running title: inhibition of glycosidase by ursolic acid**

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Article

# Tormentic Acid, a Major Component of Suspension Cells of Eriobotrya japonica, Suppresses High-Fat Diet-Induced Diabetes and Hyperlipidemia by Glucose Transporter 4 and AMP-Activated Protein Kinase Phosphorylation

Jin-Bin Wu, Yueh-Hsiung Kuo, Cheng-Hsiu Lin, Hui-Ya Ho, and Chun-Ching Shih

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# Inhibitory mechanism of two allosteric inhibitors, oleanolic acid and ursolic acid on α-glucosidase

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ABSTRACT

Glycemic control which can be efficaciously regulated by inhibiting α-glucosidase activity is an effective therapy for diabetes mellitus. This work is to investigate the kinetics and inhibition mechanism of oleanolic acid and ursolic acid on α-glucosidase. Oleanolic acid and ursolic acid exhibited potent inhibitory activities with $IC_{50}$ values of $(6.35 \pm 0.02) \times 10^{-6}$ and $(1.69 \pm 0.03) \times 10^{-5}$ mol $L^{-1}$ respectively in a reversible and non-competitive manner. Both of them binding to α-glucosidase induced the conformational change and intrinsic fluorescence quenching of α-glucosidase. The binding constants of oleanolic acid and ursolic acid with α-glucosidase at 298 K were $(2.04 \pm 0.02) \times 10^3$ and $(1.87 \pm 0.02) \times 10^3$ L $mol^{-1}$, respectively. Docking results showed that oleanolic acid and ursolic acid bound in different allosteric sites of cavity 2 and cavity 4 on α-glucosidase, respectively, which triggered allosteric regulation to perturb conformational dynamics of α-glucosidase, eventually leading to a decrease of catalytic activity of the enzyme. The substrate was not catalyzed by α-glucosidase to generate further products due to formation of a nonreactive ternary complex of oleanolic acid– or ursolic acid–α-glucosidase–substrate. The combination of oleanolic acid and ursolic acid displayed a significant synergistic inhibition on α-glucosidase.



## 1. Introduction

As one of the largest global health problems in the 21 st century, diabetes mellitus has been listed into the four main types of non-communicable diseases. Diabetes mellitus is a chronic disorder diagnosed by raised blood glucose levels and occurs when the insulin produced by the body is not enough or cannot be used [1]. In 2015, International Diabetic Foundation (IDF) reported that 1 in 11 adults suffered from diabetes (415 million) and the numbers will rise to 642 million by 2040 [1]. In 2015, diabetes has caused the deaths of 5 million people aged between 20 and 79 years, equivalent to one death every six seconds [1]. Type 2 diabetes is the main form of diabetes with the proportion of 87%–91% in high-income countries [1].

Postprandial hyperglycemia is a principal characteristic of type 2 diabetes and its complications, which can be controlled by retarding the absorption of carbohydrates [2]. α-Glucosidase situated in the small intestinal mucosal brush, degrades oligosaccharides to α-glucose by hydrolyzing α-1,4 glycosidic bond from the non-reducing end of the oligosaccharide and the product, α-glucose, retains $(\alpha \rightarrow \alpha)$ in the system [3,4]. In this case, α-glucosidase plays an important role in carbohydrates metabolism. Consequently, suppression of α-glucosidase activity by inhibitors may be an effective approach to the regulation of postprandial hyperglycemia and its complications by lessening the absorption of glucose. Considering the difficult to obtain pure α-glucosidase from mammalian, yeast α-glucosidase has been frequently used as a model for investigating the potential α-glucosidase inhibitors and the inhibitory mechanism due to its ease of access [5,6]. Yeast α-glucosidase, a retaining glycohydrolase, is a member of the sequence-related family of α-glycohydrolases (Family 13). It catalyzes the hydrolysis of the glycosidic bond with retention of the anomeric configuration via a mechanism which usually involves a covalent glycosyl-enzyme intermediate [7]. The catalytic site of α-glucosidase is composed of several glucose unit binding sites. The inhibition kinetics of α-glucosidase is consistent with Michaelis Menten kinetics, and the Lineweaver–Burk plots are often used to obtain the kinetic parameters. Some chemically synthesized

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# Maslinic acid modulates glycogen metabolism by enhancing the insulin signaling pathway and inhibiting glycogen phosphorylase

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**[ABSTRACT]**

**AIM:** To investigate the molecular signaling mechanism by which the plant-derived, pentacyclic triterpene maslinic acid (MA) exerts anti-diabetic effects.

**METHOD:** HepG2 cells were stimulated with various concentrations of MA. The effects of MA on glycogen phosphorylase a (GPa) activity and the cellular glycogen content were measured. Western blot analyses were performed with anti-insulin receptor *β* (IR*β*), protein kinase B (also known as Akt), and glycogen synthase kinase-3*β* (GSK3*β*) antibodies. Activation status of the insulin pathway was investigated using phospho-IR*β*, as well as phospho-Akt, and phospho-GSK3*β* antibodies. The specific PI3-kinase inhibitor wortmannin was added to the cells to analyze the Akt expression. Enzyme-linked immunosorbent assay (ELISA) was used to measure the effect of MA on IR*β* auto-phosphorylation. Furthermore, the effect of MA on glycogen metabolism was investigated in C57BL/6J mice fed with a high-fat diet (HFD).

**RESULTS:** The results showed that MA exerts anti-diabetic effects by increasing glycogen content and inhibiting glycogen phosphorylase activity in HepG2 cells. Furthermore, MA was shown to induce the phosphorylation level of IR*β*-subunit, Akt, and GSK3β. The MA-induced activation of Akt appeared to be specific, since it could be blocked by wortmannin. Finally, MA treatment of mice fed with a high-fat diet reduced the model-associated adiposity and insulin resistance, and increased the accumulated hepatic glycogen content.

**CONCLUSION:** The results suggested that maslinic acid modulates glycogen metabolism by enhancing the insulin signaling pathway and inhibiting glycogen phosphorylase.

**[KEY WORDS]** Maslinic acid; Insulin signal transduction; Glycogen phosphorylation a; Glycogen metabolism

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## Introduction

Abnormalities in glucose metabolism have been implicated in the etiology of several of the most common diseases affecting modern-day society, including diabetes [1], ischemic heart disease [2], stroke [3], and cancer [4]. An important component of the glucose metabolism process is glycogen metabolism, which itself plays a key role in several physiologic and pathologic processes. For example, under normal physiologic conditions, glycogen serves as an energy storage molecule, while dysfunctional glycogen metabolism can manifest as hyperglycemia [1], ischemic heart disease [2], and ischemic brain disease [3], much like dysfunctional glucose metabolism. Type 2 diabetes, in particular, is strongly associated with dysfunctional hepatic and peripheral glucose metabolism. The link between perturbed hepatic glycogen metabolism and diabetes involves its effects on insulin, which maintains blood glucose homeostasis [5].

The pentacyclic triterpenes have recently been recognized as bioactive compounds with therapeutic potential for a wide range of human diseases. Studies have demonstrated a variety of biological properties for these plant-derived compounds, including anti-inflammatory, hepatoprotective, gastroprotective, anti-ulcer, anti-viral (human immunodeficiency virus, HIV),

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Article

# Anti-diabetic effect of betulinic acid on streptozotocin-nicotinamide induced diabetic male mouse model

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Diabetes is a metabolic disease caused by abnormal insulin secretion or action. In the present study, the effects of betulinic acid (BA, a triterpene) are evaluated on glucose, α-amylase and plasma insulin levels, insulin resistance and the histopathology of pancreatic islets in streptozotocin-nicotinamide (STZ-NA) diabetic mice. Seventy adult male NMRI mice were randomly divided into seven groups: control, sham, diabetic, diabetic treated with BA (10, 20 and 40 mg/kg) and diabetic treated with metformin (200 mg/kg). Diabetes was induced in mice by intraperitoneal injection of streptozotocin 50 mg/kg after a dose of nicotinamide 120 mg/kg. Two weeks after treatment with BA, blood samples were collected for measuring glucose, α-amylase and insulin levels, and the pancreas was isolated for histopathology evaluation. Diabetes reduced the number and diameter of pancreatic islets, and increased α-amylase and insulin resistance. BA treatment reduced blood glucose, α-amylase and improved insulin sensitivity as well as pancreas histopathology. In addition, BA showed stronger effects on the pancreatic histology and insulin resistance compared to the metformin group.

**Keywords:** Betulinic acid. Diabetes. Mouse. Streptozotocin-nicotinamide.

## INTRODUCTION

Diabetes mellitus is a global health problem. Insulin deficiency leads to failure of glucose consumption in diabetes mellitus (DM) and breakdown of lipids and proteins (Mousavi *et al.,* 2011). This disease causes cardiovascular disease, retinopathy, neuropathy and other long-term complications in uncontrolled conditions (Ahangarpour *et al.,* 2016a).

In experimental studies, streptozotocin (STZ) - nicotinamide (NA) diabetic mice are one of the models that can induce mild diabetes (Tahara, Matsuyama-Yokono, Shibasaki, 2011). STZ causes pancreatic β-cell damage with transport into β-cells via the glucose transporter (Glut2) and causes DNA damage; in contrast, NA partially protects against the harmful effects of STZ (Szkudelski, 2012). Nicotinamide prevents the diabetogenic effect of STZ via the NO product and prevents apoptosis, as well as having a protective effect in the first phase of insulin release (Alenzi, 2009). Rats treated with STZ and NA manifest symptoms of type 2 diabetes, while animals with STZ-induced type 1 diabetes. β-cells in these rats were partly damaged, therefore insulin secretion was preserved in response to glucose and some other stimulants (Szkudelski, 2012). Although several therapeutic agents have been used for diabetes mellitus treatment in recent decades, most therapeutic goals have remained unmet. So, a new approach is required for treatment of type 2 diabetes. Studies have shown that to treat and manage type 2 diabetes and its complications, several long-used herbal medicines appear to be effective (Jeong *et al.,* 2012); for example, triterpenoids, which are a large group of compounds present in many plants (Silva *et al.,* 2016). These compounds divide into lupane, oleonane and ursane groups (Jager *et al.,* 2009). 3β-Hydroxy-lup-20(29)-en-28-oic acid, betulinic acid (BA) is a

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LIPIDS IN HEALTH AND DISEASE

SHORT REPORT Open Access

# Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice

Flávia Almeida Santos[1*], Julyanne Torres Frota[1], Bruno Rodrigues Arruda[1], Tiago Sousa de Melo[2], Armenio André de Carvalho Almeida da Silva[3], Gerly Anne de Castro Brito[4], Mariana Helena Chaves[3] and Vietla Satyanarayana Rao[1]

**Abstract**

**Background:** Pentacyclic triterpenes in general exert beneficial effects in metabolic disorders. This study investigated the effects of α, β-amyrin, a pentacyclic triterpene mixture from the resin of *Protium heptaphyllum* on blood sugar level and lipid profile in normal and streptozotocin (STZ)-induced diabetic mice, and in mice fed on a high-fat diet (HFD).

**Findings:** Mice treated with α, β-amyrin (10, 30 and 100 mg/kg, p.o.) or glibenclamide (10 mg/kg, p.o.) had significantly reduced STZ-induced increases in blood glucose (BG), total cholesterol (TC) and serum triglycerides (TGs). Unlike glibenclamide that showed significant reductions in BG, TC and TGs in normoglycemic mice, α, β-amyrin did not lower normal blood sugar levels but at 100 mg/kg, manifested a hypolipidemic effect. Also, α, β-amyrin effectively reduced the elevated plasma glucose levels during the oral glucose tolerance test. Moreover, the plasma insulin level and histopathological analysis of pancreas revealed the beneficial effect of α, β-amyrin in the preservation of beta cell integrity. In mice treated orally with α, β-amyrin (10, 30 and 100 mg/kg) or fenofibrate (200 mg/kg), the HFD-associated rise in serum TC and TGs were significantly less. The hypocholesterolemic effect of α, β-amyrin appeared more prominent at 100 mg/kg with significant decreases in VLDL and LDL cholesterol and an elevation of HDL cholesterol. Besides, the atherogenic index was significantly reduced by α, β-amyrin.

**Conclusions:** These findings reflect the potential antihyperglycemic and hypolipidemic effects of α, β-amyrin mixture and suggest that it could be a lead compound for drug development effective in diabetes and atherosclerosis.

**Keywords:** *Protium heptaphyllum*, α, β-amyrin, Pentacyclic triterpene, Antihyperglycemic and hypolipidemic effects, Mice

## Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Hyperglycemia and hyperlipidemia, as the most common features of diabetes mellitus, contribute to the development of microvascular and macrovascular complications of diabetes, which account for the morbidity and mortality of diabetes [1]. Search for compounds that normalize hyperglycemia, hyperlipidemia and ameliorate oxidative stress is an important objective in preventing diabetes-associated complications. None of the currently used medications reverse ongoing failure of beta cell function [2]. The search for newer drugs from natural sources, which are cost-effective and safe, without the long-term side effects may open new avenues for the treatment of diabetes and diabetes associated complications [3].

In the recent past, many pentacyclic triterpenes were shown to improve lipoprotein lipase expression, insulin sensitivity and dyslipidemia [4-7]. The resin obtained

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# Streptozotocin induced activation of oxidative stress responsive splenic cell signaling pathways: Protective role of arjunolic acid

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Chemoprevention

ABSTRACT

Present study investigates the beneficial role of arjunolic acid (AA) against the alteration in the cytokine levels and simultaneous activation of oxidative stress responsive signaling pathways in spleen under hyperglycemic condition. Diabetes was induced by injection of streptozotocin (STZ) (at a dose of 70 mg/kg body weight, injected in the tail vain). STZ administration elevated the levels of IL-2 as well as IFN-γ and attenuated the level of TNF-α in the sera of diabetic animals. In addition, hyperglycemia is also associated with the increased production of intracellular reactive intermediates resulting with the elevation in lipid peroxidation, protein carbonylation and reduction in intracellular antioxidant defense. Investigating the oxidative stress responsive cell signaling pathways, increased expressions (immunoreactive concentrations) of phosphorylated p65 as well as its inhibitor protein phospho IκBα and phosphorylated mitogen activated protein kinases (MAPKs) have been observed in diabetic spleen tissue. Studies on isolated splenocytes revealed that hyperglycemia caused disruption of mitochondrial membrane potential, elevation in the concentration of cytosolic cytochrome *c* as well as activation of caspase 3 leading to apoptotic cell death. Histological examination revealed that diabetic induction depleted the white pulp scoring which is in agreement with the reduced immunological response. Treatment with AA prevented the hyperglycemia and its associated pathogenesis in spleen tissue. Results suggest that AA might act as an anti-diabetic and immunomodulatory agent against hyperglycemia.



## Introduction

In modern civilization diabetic mellitus is an increasing health problem. It is a group of metabolic diseases characterized by hyperglycemia resulting from defective insulin secretion or resistance to insulin action or both (Braun et al., 1995). Several lines of earlier investigations have examined that oxidative stress plays an important role in the etiology of diabetes as well as associated complications (Baynes, 1991; Nogichi, 2007; Oberley, 1988). Various free radicals and non radical species react with several amino acid residues altering their structures and by extension, the tertiary structures of the protein molecules. Immune deficiencies, especially cell-mediated immunity, have been suggested as a major contributor to infection and morbid complication of diabetes patients (Geerlings and Hoepelman, 1999; Chang, and Shaio, 1999). Spleen is one of the principle sites for the initiation of most primary immune responses, for B lymphocyte activation and the production of antibodies. β-Cell destruction in type 1 diabetes probably involves cytokine production by spleen cells (Thorvaldson et al., 2003). The balance between cytokines produced by T helper 1 and 2 (Th1 and Th2) lymphocytes has been postulated to influence the outcome of diabetes. In type 1 diabetes, Th1 response (a cytokine production dominated by a group of cytokines, e.g. TNF-α, IFN-γ, IL-1β and IL-12) has been suggested to be connected with a β-cell destructive insulitis; however, Th2 type response (production of IL-4 and IL-10) is associated with a non-destructive insulitis (Kolb, 1997). The altered cytokine production, in addition, also caused activation of redox-sensitive transcription factors (TFs), nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). A variety of agents which are known to generate ROS, have also been shown to regulate AP-1 activation (Hsu et al., 2000; Klaunig and Kamendulis, 2004) and that could be regulated by the activation of mitogen activated protein kinases (MAPKs). Streptozotocin (STZ), an antibiotic, produced by *Streptomyces achromogenes*, is the most commonly used agent in experimental diabetes due to its ability to destruct pancreatic β-islets cells possibly via the formation of

*Abbreviations:* AA, arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid); CAT, catalase; DAB, 3,3'-diaminobenzidine tetrahydrochloride; DMEM, Dulbecco's modified eagle's medium; FACS, fluorescence activated cell sorting; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione-S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; M2VP, 1-methyl-2-vinylpyridinium trifluoromethanesulfonate; MAPKs, mitogen-activated protein kinases; MDA, malonaldehyde; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase.

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RESEARCH ARTICLE

# Prunin is a highly potent flavonoid from *Prunus davidiana* stems that inhibits protein tyrosine phosphatase 1B and stimulates glucose uptake in insulin-resistant HepG2 cells

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**Abstract** Prunin is the main flavonoid in *Prunus davidiana* stems and improves hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats. The aim of this study was to investigate the in vitro anti-diabetic potential of prunin via the inhibition of protein tyrosine phosphatase 1B (PTP1B), α-glucosidase, peroxynitrite ($ONOO^-$)-mediated tyrosine nitration, and stimulation of glucose uptake in insulin-resistant hepatocytes. In addition, a molecular docking simulation was performed to predict specific prunin binding modes during PTP1B inhibition. Prunin showed strong inhibitory activity against PTP1B, with an $IC_{50}$ value of $5.5 \pm 0.29$ μM, and significant inhibitory activity against α-glucosidase, with an $IC_{50}$ value of $317 \pm 2.12$ μM. Moreover, a kinetics study revealed that prunin inhibited PTP1B ($K_i = 8.66$) and α-glucosidase ($K_i = 189.56$) with characteristics typical of competitive and mixed type inhibitors, respectively. Docking simulations showed that prunin selectively inhibited PTP1B by targeting its active site and exhibited good binding affinity, with a docking score of −9 kcal/mol. Furthermore, prunin exhibited dose-dependent inhibitory activity against $ONOO^-$-mediated tyrosine nitration and stimulated glucose uptake by decreasing PTP1B expression level in insulin-resistant HepG2 cells. These results indicate that prunin has significant potential as a selective PTP1B inhibitor and may possess anti-diabetic properties by improving insulin resistance.

**Keywords** Prunin · Protein tyrosine phosphatase 1B · Anti-diabetic · Glucose uptake · HepG2 cell · Molecular docking

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## Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder and has become one of the world's most serious health concerns (Rengasamy et al. 2013). According to the World Health Organization, approximately 350 million people suffer from DM and this may double by 2030 (Pantidos et al. 2014). Type 2 diabetes mellitus (T2DM) is the most common form of DM and is characterized by high glucose levels and relative insulin deficiency (Guo et al. 2015). Without proper treatment, T2DM can cause severe secondary complications, including atherosclerosis, renal dysfunction and failure, cardiac abnormalities and ocular disorders (Wang et al. 2015). Thus, the development of new anti-diabetic agents is an urgent need, together with adequate therapeutic approaches to control this disease. Several potentially effective therapeutic targets, such as protein tyrosine phosphatase (PTP1B), and α-glucosidase, have been identified (Kokil et al. 2015). PTP1B is an intracellular PTP expressed in insulin responsive tissues (Forsell et al. 2000) that blocks insulin signaling by dephosphorylating the insulin receptor and its substrate (Popov 2011). The enzyme also works as a negative regulator of the leptin signaling pathway and is involved in food intake and peripheral energy expenditure, and the GLUT 4 transporter involved in glucose uptake (Morris and Rui 2009). PTP1B knockout murine models exhibit

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ORIGINAL PAPER

# Antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin–nicotinamide-induced experimental diabetic rats

**T. Annadurai · A. R. Muralidharan · T. Joseph · M. J. Hsu · P. A. Thomas · P. Geraldine**

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**Abstract** In the present study, the putative antihyperglycemic and antioxidant effects of a flavanone, naringenin, were evaluated in comparison with those of glyclazide, a standard drug for therapy of diabetes mellitus. Diabetes was induced experimentally in 12-h-fasted rats by intraperitoneal injections of first streptozotocin (50 mg/kg b.w.) and then of nicotinamide (110 mg/kg b.w.) after a 15-min interval. Untreated diabetic rats revealed the following in comparison with normal rats: significantly higher mean levels of blood glucose and glycosylated hemoglobin, significantly lower mean levels of serum insulin, significantly lower mean activities of pancreatic antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase), significantly lower mean levels of plasma non-enzymatic antioxidants (reduced glutathione, vitamin C , vitamin E), significantly elevated mean levels of pancreatic malondialdehyde (MDA) and significantly elevated mean activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Following oral administration of naringenin (50 mg/kg b.w./day) to diabetic rats for 21 days, the following observations were made in comparison with untreated diabetic rats: significantly lower mean levels of fasting blood glucose and glycosylated hemoglobin, significantly elevated serum insulin levels, significantly higher mean activities of pancreatic enzymatic antioxidants, significantly higher mean levels of plasma non-enzymatic antioxidants, lower mean pancreatic tissue levels of MDA and lower mean activities of ALT, AST, ALP and LDH in serum. The values obtained in the naringenin-treated animals approximated those observed in glyclazide-treated animals. Histopathological studies appeared to suggest a protective effect of naringenin on the pancreatic tissue in diabetic rats. These results suggest that naringenin exhibits antihyperglycemic and antioxidant effects in experimental diabetic rats.

**Keywords** Diabetes · Naringenin · Streptozotocin–nicotinamide · Oxidative stress

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## Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to insufficiency of

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Research Paper

# Hepatoprotective potential of isoquercitrin against type 2 diabetes-induced hepatic injury in rats

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## ABSTRACT

**Non-alcoholic fatty liver disease is a main complication of type 2 diabetes. Isoquercitrin are employed for antidiabetic therapies, but the effects on liver function and the hepatocytes are unclear. The aim of this study was to investigate the effects of isoquercitrin on the T2DM-induced hepatic injury in rats. Isoquercitrin (10 mg/kg/d, 30 mg/kg/d), sitagliptin phosphate (10 mg/kg/d) was given orally for 21 days. The administration of isoquercitrin at 10 mg/kg/d and 30 mg/kg/d showed a dose dependent. Compare to the negative control (treated with saline), rats medicated with isoquercitrin (30 mg/kg/d) and sitagliptin phosphate (10 mg/kg/d) improved the clinical symptoms, FBG and glucose tolerance, reduced serum ALT, AST and IR, but increased TP, Alb, SOD, GSH, MDA, HDL-C, INS and GLP-1. On histology, Rats of these to groups presented nearly normal liver tissue and Langerhans, degeneration, necrosis and apoptosis were markedly reduced. Instead, hepatocytes showed regenerate. These two groups also showed significant increase in mRNA expression of PKA, AKT, PKCa, InsR and PI3K, and a decrease in DPP-IV mRNA level. These results indicated that treatment with isoquercitrin protects against hepatic injury by T2DM.**

## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia (fasting state>7 mmol/L). Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounts for 90% of the patients. The mechanisms of T2DM are the insulin resistance (IR) of liver and muscle. Thus nearly all T2DM patients manifested severe hepatic steatosis [1].

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathologic syndrome, recognized as one of the most common causes of liver damage, including hepatic steatosis [2]. Alterations in hepatic metabolism lead to overproduction of glucose and lipids, which in turn abet development of glucose intolerance and dyslipidemias to induce T2DM [3]. Patients with NAFLD increased about 5-fold of the incidence of T2DM [4]. While T2DM are the strongest predictors of NAFLD, more than 70% patients suffered NAFLD [5].

Isoquercitrin (quercetin-3-O-b-D-glucopyranoside, $C_{20}H_{21}O_{12}$) is a wildly existed natural flavonoids in plants [6]. It possess various pharmacological activities, such as antioxidant [7], anti-inflammatory [8], anti-cancer [9]. Previous research has reported that isoquercitrin improved

Article

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# Modulatory effect of baicalein on gene expression and activity of antioxidant enzymes in streptozotocin-nicotinamide induced diabetic rats

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Oxidative stress plays the central role in the pathogenesis and progression of diabetic complications. The present study aims to investigate the beneficial effect of oral administration of flavone baicalein in streptozotocin-nicotinamide (STZ-NA) induced diabetic rats by measuring oxidative stress markers, antioxidant enzyme activities and expression analysis of antioxidant genes. Experimental diabetes was induced by a single intraperitoneal (i.p.) injection of STZ (55 mg /kg b.wt), 15 min after the i.p. administration of NA. At the end of the experimental period, thiobarbituric acid reactive substances (TBARS), activities of antioxidant enzymes and expression levels of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) were measured in diabetic rats along with serum biochemical parameters namely total cholesterol (TC), total triglyceride (TG), aspartate transaminase (AST) alanine transaminase (ALT) and glycosylated hemoglobin (HbA1c). Oral administration of baicalein (40 mg/kg b.wt/day) demonstrated a significant ameliorative effect on all studied biochemical and oxidative stress parameters. Biochemical findings were corroborated by qPCR expression analysis which showed significant upregulation of antioxidant genes in diabetic rats. These results suggest that baicalein supplementation may reduce diabetes and its complications by suppressing oxidative stress and enhancing gene expression and antioxidant enzyme activities in diabetic rats.

**Keywords:** Diabetes. Streptozotocin/pharmacology. Baicalein/pharmacology. Nicotinamide/pharmacology. Glibenclamide. Diabetes Mellitus/ Experimental/prevention and control. Gene Expression/drug effects. Antioxidants/pharmacology. Glyburide/pharmacology. Rats.

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated levels of glucose in the blood (hyperglycemia) and insufficiency of production or action of insulin produced by the pancreas in our body (Maritim, Sanders, 2003). Diabetes is currently affecting more than 100 million people worldwide. Oxidative stress plays a pivotal role in the onset and progression of diabetic complications (Ayepola, Brooks, Oguntibeju, 2014). Oxidative stress results from an imbalance between the production and neutralization of reactive oxygen species (ROS) such as highly reactive hydroxyl radicals, superoxide anion, peroxyl radicals, singlet oxygen, peroxynitrite, and hydrogen peroxide (Sellamuthu *et al.*, 2013). Oxidative stress-induced complications from diabetes include neuropathy, coronary artery disease, nephropathy, retinopathy (Phillips *et al.*, 2004). There are several reports on altered antioxidant defenses and the role of free radicals in the etiology of diabetes and its complications (Raza *et al.*, 2011). While the external supply of insulin and other medications can control many aspects of diabetes, numerous complications that affect the kidney, peripheral nerves, vascular system, retina, lens, and skin are common and are extremely important factors in terms of longevity and quality of life (Maritim, Sanders, 2003). Increased oxidative stress is a key participant in the development and progression of diabetes and different diabetes-related complications (Giacco, Brownlee, 2010). It is very important to explore the relationship between free radicals, diabetes, and its complications, and to reveal the mechanisms by which increased oxidative stress accelerates the development of diabetic complications, in an effort to find effective treatment options.

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# Accepted Manuscript

Baicalein improves glucose metabolism in insulin resistant HepG2 cells

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Original article

# Modulatory effect of vanillic acid on antioxidant status in high fat diet-induced changes in diabetic hypertensive rats

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ABSTRACT

The worldwide incidence of diabetes has increased dramatically along with widespread lifestyle and dietary changes. Diets high in fat are strongly associated with the development of obesity and can induce insulin resistance in humans and animals. It is clear that obesity constitutes a risk factor for contributing to the development of type 2 diabetes. In the present study, we investigated the therapeutic potential action of vanillic acid on diabetes associated complications using a rat model. Rats were made diabetic hypertensive by high fat diet (HFD) for 20 weeks and were treated with vanillic acid (50 mg/kg bw) for last 8 weeks. The effects of vanillic acid on glucose, plasma insulin, systolic and diastolic blood pressure, thiobarbituric acid reactive substances (TBARS), hydroperoxides as a lipid peroxidation marker, and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), vitamin C and vitamin E as an antioxidant marker, AST and ALT as a liver function marker, urea, uric acid and creatinine as a kidney function marker were investigated. Histopathology of liver and kidney was also investigated as part of the pathology of diabetes. Treatment of diabetic rats with oral administration of vanillic acid at a dose of 50 mg kg/body weight for 8 weeks resulted in a significant decrease in fasting plasma glucose, insulin and blood pressure levels in comparison with diabetic control group. The antioxidant activities were significantly increased and the levels of lipid peroxidation markers were significantly decreased in diabetic hypertensive rats treated with vanillic acid. These results suggest that vanillic acid offer a modulatory effect on control of diabetic hypertension by reduction of blood glucose, insulin and blood pressure, combating oxidative stress by activation of tissue antioxidants.



## 1. Introduction

Diabetes mellitus and hypertension are interconnected diseases that strongly predispose an individual to atherosclerotic cardiovascular disease. Hypertension is about twice as frequent in persons with diabetes as in those without [1,2]. Lifestyle and genetic factors are important factors contributing to both hypertension and diabetes mellitus. The prevalence of coexisting hypertension and diabetes appears to be increasing in industrialized nations because populations are aging and both hypertension and NIDDM incidence increases with age [2]. Data obtained from death certificates show that hypertensive disease has been implicated in 4.4% of deaths coded to diabetes, and diabetes was involved in 10% of deaths coded to hypertensive disease. Indeed, an estimated 35% to 75% of diabetic cardiovascular and renal complications can be attributed to hypertension [1]. For all these reasons, hypertension and diabetes should be recognized and treated early and aggressively. Essential hypertension accounts for the majority of hypertension in individuals with diabetes, particularly those with NIDDM [type II diabetes], who constitute more than 90% of people with a dual diagnosis of diabetes and hypertension [1,2].

Oxidative stress constitutes a unifying mechanism of injury in many types of vascular diseases. It has been reported that hyperglycemia increases oxidative stress through overproduction of reactive oxygen radicals [ROSs] [3–5]. Free radicals are very reactive chemical species, can cause oxidative injury to the living beings by attacking the macromolecules like lipids, carbohydrates, proteins and nucleic acids. There is much evidence concerning the contribution of ROS molecules to organ injury in systems, such as heart, liver, and central nervous system [6–8], as well as that oxidative damage is increased in diabetes [9]. On the other hand, it

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# Antihyperglycaemic potential of rosmarinic acid attenuates glycoprotein moiety in high-fat diet and streptozotocin-induced diabetic rats

Sundaram Ramalingam, Muthu Karuppiah & Muthusamy Thiruppathi

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**Original article**

# The anti-inflammatory and anti-glycative effects of rosmarinic acid in the livers of type 1 diabetic mice

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*Keywords:*
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**ABSTRACT**

Background: Rosmarinic acid (RA) is a polyphenol present in members of the *Lamiaceae* family. In this study, yhe anti-inflammatory and anti-glycative effects of RA in the livers of type 1 diabetic mice were examined.

Methods: The diabetic mice were divided into three groups: diabetic mice with 0, low dose RA (25 mg/*ml*), and high dose RA (50 mg/*ml*). One group of non-diabetic mice was used as a control for comparison. RA was supplied *via* daily 200 μL oral injections for 9 weeks. The level of interleukin (IL)-6, the tumor necrosis factor (TNF)-alpha, the prostaglandin $E_2$ ($PGE_2$), and the activity of cyclooxygenase (COX)-2 in the livers were measured. The hepatic receptor of advanced glycative endproduct (RAGE), the sorbitol levels, and the glyoxalase 1 (GLO-1) activity were also determined.

Results: Compared with diabetic group that received no RA, the groups with RA supplements at both levels of dosages had increased body weight and had both decreased water intake and feed intake ($p$<0.05). RA intake was found to reduce plasma glucose level and elevate plasma insulin level when compared with the diabetic group that received no RA ($p$<0.05). RA treatments lowered the hepatic level of IL-6, TNF-alpha, and $PGE_2$, as well as the activity of COX-2 ($p$<0.05). RA administration also decreased hepatic RAGE and sorbitol levels, and GLO-1 activity when compared with the diabetic group that received no RA ($P$<0.05).

Conclusion: These findings support the conclusion that rosmarinic acid (RA) could be a potent protective agent for the liver against diabetic injury..

## 1. Introduction

Rosmarinic acid (RA) is a main polyphenol present in *Rosmarinus Officinalis* L., Coleus aromaticus, and members of the Lamiaceae family. It has been documented that RA has many bio-activities including anti-oxidative, anti-microbial, anti-inflammatory, anti-metastatic, neuroprotective, and immunomodulatory effects [1-3]. Furthermore, RA has been considered to be a potent agent for chronic disease prevention and/or alleviation [3-5]. The anti-diabetic effects of RA in rodents have been examined, and the authors of those studies have indicated that RA could improve glycemic control, oxidative stress and vascular dysfunction, which in turn can attenuate the progression of diabetes, as well as delay the occurrence of diabetic complications [6-8].

Inflammation and glycation are two major pathological characteristics of diabetes types 1 and 2. Although the anti-inflammatory effect of RA has been reported [9], it is still unclear whether RA intake could alleviate hepatic inflammation under diabetic conditions. The liver preserves many nutrients and exerts many crucial physical functions. If RA does protect the liver against diabetes-related inflammation, it may be a beneficial nutritional support for diabetic subjects. Thus, in our present study we examined the impact of RA upon a variety of inflammatory factors including interleukin (IL)-6, prostaglandin $E_2$ ($PGE_2$), and cyclooxygenase (COX)-2 in the livers of type 1 diabetic mice to evaluate the anti-inflammatory effects of RA. Thus far, less attention has been paid to the anti-glycative activities of RA, especially in the liver. The increase in the receptors of advanced glycative endproducts (RAGE) plays an important role in diabetic progression because RAGE reacts with AGEs and other ligands such as beta-amyloid, and the interactions of RAGE and its ligands further activates other signal pathways responsible for the production of oxidative, inflammatory, or angiogenic factors [10-12]. Thus, the decline of RAGE formation due to RA definitely contributes to diminishing glycative injury and other diabetic pathological stress. In addition, sorbitol level and glyoxalase 1 (GLO-1) activity are two biomarkers used for evaluating glycative stress. If RA decreases sorbitol generation and/or increases GLO-1 activity, it subsequently may alleviate glycative injury. In our present study, type 1 diabetes was induced in mice, and followed with RA treatment. The anti-inflammatory and anti-glycative effects of RA in the livers of these mice was examined.

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# Antioxidant and antihyperlipidaemic activity of protocatechuic acid on streptozotocindiabetic rats

Ranganathan Harini & Kodukkur Viswanathan Pugalendi

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Endocrine Pharmacology

# Antihyperglycemic activity and antidiabetic effect of methyl caffeate isolated from *Solanum torvum* Swartz. fruit in streptozotocin induced diabetic rats

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ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternatives to treat diabetes mellitus. *Solanum torvum* Swartz. fruit is widely used in the traditional system of medicine to treat diabetes. In the present study methyl caffeate, isolated from *S. torvum* fruit, was screened for its efficacy in controlling diabetes in animal models. Antihyperglycemic effect of methyl caffeate was studied in normal glucose-fed rats. The effects of oral administration of methyl caffeate (10, 20 and 40 mg/kg) for 28 days on body weight, fasting blood glucose, plasma insulin, hemoglobin, glycated hemoglobin, total protein, hepatic glycogen and carbohydrate metabolism enzymes in streptozotocin induced diabetic rats were investigated. Histological observations in the pancreas and GLUT4 expression in skeletal muscles were also studied. Methyl caffeate at 40 mg/kg significantly prevented the increase in blood glucose level after glucose administration at 60 min in comparison to the hyperglycemic control group. In streptozotocin induced diabetic rats, methyl caffeate produced significant reduction in blood glucose and increased body weight. The levels and/or activities of other biochemical parameters were near normal due to treatment with methyl caffeate. Methyl caffeate treated diabetic rats showed upregulation of GLUT4 and regeneration of β-cells in the pancreas. These results substantiated that methyl caffeate possessed hypoglycemic effect, and it could be developed into a potent oral antidiabetic drug.



## 1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia that involves abnormalities in both insulin secretion and action at peripheral tissues (Tian et al., 2003). The high concentration of blood glucose and other biochemical abnormalities results from a deficiency of insulin and/or from a subsensitivity to insulin in target cells (Annapurna et al., 2001). This includes impairment in the insulin signaling pathway leading to a failure of the insulin stimulated glucose uptake through GLUT4 (Glucose transporter 4) in targeted tissues like muscle and fat. In muscle cells, this is due to the inability of the insulin to stimulate the translocation of GLUT4 to the plasma membrane leading primarily to hyperglycemia (Sujatha et al., 2010). According to the World Health Organization (WHO), diabetes mellitus is an ever-increasing disease (WHO, 2006). The disease affects all components of the world but the percentages of diabetics in global population are particularly high in Asia and Europe (World Diabetes Foundation (WDF), 2010). The practice of herbal medicine to treat diabetes mellitus is almost universal among non-industrialized societies (Singh et al., 2001). The WHO estimated that 80% of the diabetic people in the world's population presently depend upon herbal medicine for their successive treatments (WHO, 2008). Antidiabetic agents from medicinal plants could serve as a good source for drug design and much attention has been fixed on formulations of herbal medicine (Vishwakarma et al., 2010).

*Solanum torvum* Swartz. (Solanaceae) is a diminutively minuscule shrub distributed widely in South India, Malaya, China, Philippines and Tropical America. Its edible fruits, commonly available in the markets, are utilized as vegetable and are regarded as essential ingredients in the South Indian population's diet. Pharmacological studies on this fruit demonstrated antidiabetic (Gandhi et al., 2011), antimicrobial, antioxidant, antiviral, immuno-secretory, analgesic, anti-inflammatory, cardiovascular and anti-platelet aggregation activities (Mohan et al., 2009). Chemical constituents reported from the *S. torvum* fruit include isoflavonoid sulfate, steroidal glycosides, chlorogenone, neochlorogenone, triacontane derivatives, 22-β-O-spirostanol oligoglycosides, 26-O-β-glucosidase, rutin, caffeic acid, gallic acid, catechin, pyrogallol (Kusirisin et al., 2009; Mohan et al., 2009) and methyl caffeate (Takahashi et al., 2010).

Methyl caffeate isolated from the *S. torvum* fruit possessed α-glucosidase inhibition activity in rat intestine (Takahashi et al., 2010). The protective efficacy of methyl caffeate has been reported in cellular models of oxidative stress (Ishige et al., 2001).

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# Ferulic acid alleviates the symptoms of diabetes in obese rats

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ABSTRACT

The effect of ferulic acid on biochemical and histological properties, mRNA expression of HO-1 and GST in the liver and heart were investigated in obese, diabetes rats after ferulic acid administration for 16 weeks. The results showed that ferulic acid (60 mg/kg) decreased the activities of ALT, AST, CK, and LDH in the serum, by 53.5, 33.6, 47.8, and 405.5% respectively; and reduced cell apoptosis from 16.55 to 9.11% in the liver, and 11.27 to 5.09% in the heart. Ferulic acid significantly increased the antioxidant activity in the plasma, liver, and heart, and upregulated the mRNA expression of HO-1 and GST in the cells of liver and heart of the diabetes animals. Moreover, ferulic acid maintained the body weight, significantly decreased serum glucose and lipid, and advanced glycation end products in the plasma, liver, and heart. It can be concluded that ferulic acid can alleviate late-stage diabetes in obese rats.



## 1. Introduction

Diabetes mellitus (DM) is a major public health burden worldwide. In 2011, 365 million people in the world had diabetes, 90% of which were cases with type 2 diabetes mellitus (T2DM) (Scully, 2012). According to recent estimates, more than 20 million people in the United States and 90 million people in China have T2DM (Styskal, Van Remmen, Richardson, & Salmon, 2012). T2DM caused more than 3.5 million deaths in middle-income countries in 2011 (Scully, 2012; Styskal et al., 2012).

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*Abbreviations:* AGEs, advanced glycation end products; ALT, alanine aminotransferase; AST, aspartate aminotrnasferase; BUN, blood urea nitrogen; CK, creatine kinase; DMBG, dimethylbiguanide; FA, ferulic acid; FINS, fasting insulin; FPG, fasting plasma glucose; GSH, glutathione; GST, glutathione S-transferase; HDL-C, high density lipoprotein-cholesterol; HE, hematoxylin-eosinstaiing; HO-1, heme oxygenase-1; LDH, lactate dehydrogenase; LDL-C, low density lipoprotein-cholesterol; PBS, phosphate buffered saline; SOD, superoxide dismutase; STZ, streptozocin; T2DM, type 2 diabetic mellitus; TBARS, thiobarbituric acid-reactive substances; TC, total cholesterol; TG, triglyceride; Tunel, transferase dUTP nick-end labeling

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*Research Article*

# Evaluation of α-Glucosidase Inhibitory Effect of 50% Ethanolic Standardized Extract of *Orthosiphon stamineus* Benth in Normal and Streptozotocin-Induced Diabetic Rats

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In the present study, a 50% ethanolic extract of *Orthosiphon stamineus* was tested for its α-glucosidase inhibitory activity. *In vivo* assays of the extract (containing 1.02%, 3.76%, and 3.03% of 3′hydroxy-5,6,7,4′-tetramethoxyflavone, sinensetin, and eupatorin, resp.) showed that it possessed an inhibitory activity against α-glucosidase in normal rats loaded with starch and sucrose. The results showed that 1000 mg/kg of the 50% ethanolic extract of *O. stamineus* significantly ($P < 0.05$) decreased the plasma glucose levels of the experimental animals in a manner resembling the effect of acarbose. In streptozotocin-induced diabetic rats, only the group treated with 1000 mg/kg of the extract showed significantly ($P < 0.05$) lower plasma glucose levels after starch loading. Hence, α-glucosidase inhibition might be one of the mechanisms by which *O. stamineus* extract exerts its antidiabetic effect. Furthermore, our findings indicated that the 50% ethanolic extract of *O. stamineus* can be considered as a potential agent for the management of diabetes mellitus.

## 1. Introduction

Type 2 diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia, a condition which could either be attributed to insufficient insulin secretion or insulin resistance. The number of diabetic patients is rapidly rising in most parts of the world, especially in the developing countries such as Thailand, India, and Indonesia. Controlling blood glucose levels of diabetics within the normal range is mainly based on the use of oral hypoglycemic/antihyperglycemic agents and insulin. However, these conventional treatments have undesirable side effects [1–3]. Those shortcomings have led to a great interest in the use of medicinal plants as alternatives for the management of type 2 diabetes mellitus [4]. Control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications. Basically, a sudden rise in blood glucose levels, causing hyperglycemia in type 2 diabetic patients, would be due to starch hydrolysis by the α-amylase and α-glucosidases found in gastrointestinal tract [5]. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides (glucose and fructose) before they can be transported across the intestinal lumn (mainly in duodenum and upper jejunum) into the bloodstream and thereby increase blood glucose level. Thus, one of the effective strategies for the management of blood glucose level in type 2 DM is by inhibition of α-glucosidases and α-amylase [6, 7] which reduces the digestion of carbohydrates for production of monosaccharide and, hence, indirectly decreases blood glucose level. Among glucose lowering medications, α-glucosidase inhibitors delay the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks [8]. It was demonstrated that α-glucosidase inhibitors could be used to prevent disorders such as diabetes, obesity, hyperlipidaemia, and hyperlipoproteinaemia [9]. Our previous study showed that 50% ethanolic extract of *O. stamineus* and its active ingredient, sinensetin, were able to inhibit α-glucosidase and α-amylase *in vitro* [10]. To the best of our knowledge, there have been no other reports on *in vivo* α-glucosidase inhibitory activity of *O. stamineus*. The present

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# Hexane Extract of *Orthosiphon stamineus* Induces Insulin Expression and Prevents Glucotoxicity in INS-1 Cells

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**Background:** Hyperglycemia, a characteristic feature of diabetes, induces glucotoxicity in pancreatic β-cells, resulting in further impairment of insulin secretion and worsening glycemic control. Thus, preservation of insulin secretory capacity is essential for the management of type 2 diabetes. In this study, we evaluated the ability of an *Orthosiphon stamineus* (OS) extract to prevent glucotoxicity in insulin-producing cells.
**Methods:** We measured insulin mRNA expression and glucose-stimulated insulin secretion (GSIS) in OS-treated INS-1 cells after exposure to a high glucose (HG; 30 mM) concentration.
**Results:** The hexane extract of OS elevated mRNA expression of insulin as well as pancreatic and duodenal homeobox-1 of INS-1 cells in a dose-dependent manner. The hexane OS extract also increased the levels of phosphorylated phosphatidylinositol 3-kinase (PI3K) in a concentration-dependent manner. Additionally, Akt phosphorylation was elevated by treatment with 100 and 200 μmol of the hexane OS extract. Three days of HG exposure suppressed insulin mRNA expression and GSIS; these expressions were restored by treatment with the hexane OS extract. HG elevated peroxide levels in the INS-1 cells. These levels were unaffected by OS treatment under both normal and hyperglycemic conditions.
**Conclusion:** Our results suggested that the hexane extract of OS elevates insulin mRNA expression and prevents glucotoxicity induced by a 3-day treatment with HG. This was associated with the activation of PI-3K and Akt.

**Keywords:** Glucose-stimulated insulin secretion, Insulin mRNA; Glucotoxicity; *Orthosiphon stamineus*

## INTRODUCTION

Hyperglycemia is a key pathologic feature of type 2 diabetes that mainly results from insulin resistance and pancreatic β-cell dysfunction. However, insulin resistance alone does not induce hyperglycemia if compensatory insulin secretion is maintained. When insulin secretion is not sufficient to overcome insulin resistance, hyperglycemia develops. Moreover, elevated glucose concentrations worsen defective insulin secretion. This condition is known as "glucotoxicity" [1-4]. Thus, β-cell preservation is essential for the prevention and management of type 2 diabetes.

Insulin is secreted through an exocytotic process that releases insulin granules following the influx of calcium ions through voltage-dependent calcium channels. This results from the closure of ATP-sensitive potassium channels [5] or the activation of the phospholipase C/phosphatidyl 4,5-biphosphate/diacylglycerol/protein kinase C pathway in response to glucose or other insulin secretagogues [6-8]. Additionally, insulin gene expression is stimulated by glucose or signaling molecules through the activation of transcriptional factors, such as pancreatic duodenal homeobox-1 (PDX-1) and musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) [9]. The expressions of PDX-1 and MafA may be associated with the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in insulin-producing cells [10,11]. In general, chronic hyperglycemia suppresses both insulin mRNA expression and glucose-induced insulin secretion [4]. Thus, pro-

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Original Paper

Thieme

# Antiobesity and Lipid Lowering Effects of *Orthosiphon stamineus* in High-Fat Diet-Induced Obese Mice

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**Key words**
*Orthosiphon stamineus*, Lamiaceae, rosmarinic acid, obesity, hypolipidemic, high-fat diet

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## ABSTRACT

The present study investigated the antiobesity and lipid lowering effects of an ethanolic extract of leaves obtained from *Orthosiphon stamineus* (200 and 400 mg/kg) and its major compound (rosmarinic acid, 10 mg/kg) in obese mice (C57BL/6) induced by a high-fat diet. Continuous supplementation with *O. stamineus* extract (200 and 400 mg/kg) for 8 weeks significantly decreased body weight gain ($p < 0.05$). However, supplementation with rosmarinic acid, a constituent in the extract, produced only a slight reduction in body weight gain compared to the high-fat diet control group. Food intake between the treatment and the high-fat diet groups was similar, which suggested that the plant extract did not suppress food intake. Further, body weight reduction of the treatment groups was not due to a decreased reduction in energy intake. Compared to the high-fat diet-fed group, serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol levels were significantly reduced in the treated groups, while high-density lipoprotein cholesterol levels were not significantly altered. Accumulation of hepatic lipid droplets induced by a high-fat diet was markedly inhibited by *O. stamineus* extract. In addition, *O. stamineus* significantly diminished liver malondialdehyde production, and significantly elevated the activities of hepatic superoxide dismutase. The present study showed that an ethanolic extract prepared from the leaves of *O. stamineus* can significantly reduce a gain in body weight, enhance antioxidant activity, and possess hypolipidemic and antiobesity effects, thereby protecting against the adverse effects of high-fat diet-induced obesity.



## Introduction

Obesity, which has been termed the "New World Syndrome", is now considered a global problem by the WHO and is associated with numerous chronic disabilities and diseases, such as dyslipidemia, fatty liver disease, osteoarthritis, hypertension, obstructive sleep apnea, gallstones, type 2 diabetes, reproductive and gastrointestinal cancers, coronary artery disease, heart failure, and stroke [1]. It has been defined as an excessive amount of body fat that can enhance the risk of medical ailments and premature death. The chronic imbalance between energy intake and energy expenditure has been considered the most significant factor that leads to obesity [2]. On a global scale, obesity has reached epidemic proportions and is a major contributor to the global burden of chronic disease and disability. According to the WHO, obesity kills more people than those that are underweight, and 65 % of the population who live in developed countries are overweight or obese [3]. In the last few years, most of the antiobesity drugs that were approved and marketed have now been withdrawn due to serious adverse effects [4]. Orlistat, which is considered a potent inhibitor of gastric, pancreatic and carboxyl ester lipases has been proven to be effective for long-term treatment of obesity [5]. However, it has been reported that orlistat is associated with vari-

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# Effects of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats

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**Abstract**

The objective of this study was to investigate the effects of *Orthosiphon stamineus* Benth. aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. The chemical screening of the extract showed phenolic compound and flavonoid content were 13.24 ± 0.33 mg/g and 1.73 ± 0.14 μg/g, respectively. In oral glucose tolerance test, the extract (0.2–1.0 g/kg) significantly decreased plasma glucose concentration in a dose-dependent manner in both normal and diabetic rats. The extract at 1.0 g/kg was most effective in decreasing plasma glucose concentrations and the response was closed to the result of glibenclamide (5 mg/kg). After repeated daily oral administrations of the extract (0.5 g/kg) for 14 days, the extract significantly reduced plasma glucose concentration in diabetic rats at days 7 and 14. By the end of the study, plasma triglyceride concentration was lower in the extract-treated diabetic rats than untreated ones. Furthermore, plasma HDL-cholesterol concentration was significantly increased in diabetic rats treated with the extract. In perfused rat pancreas, the extract did not increase insulin secretion in the presence of 5.5 mM glucose, but 100 μg/ml extract potentiated glucose-induced insulin secretion. Our findings suggested that *Orthosiphon stamineus* aqueous extract is effective for alleviating hyperglycemia and improving lipid profile in diabetic rats.


*Keywords:* *Orthosiphon stamineus*; Plasma glucose; Diabetes; Lipid profile

## 1. Introduction

Type 2 diabetes mellitus is a metabolic disease of hyperglycemia, a condition caused either by insufficient insulin secretion or insulin resistance. The number of diabetic patients is rapidly rising in most parts of the world, especially in developing countries such as Thailand, India, and Indonesia. In general, the control of blood glucose concentrations near normal range in patients is mainly based on the use of oral hypoglycemic/anti-hyperglycemic agents and insulin. However, all of these treatments have limited efficacy and associated with undesirable side effects (Harrower, 1994; Reuser and Wisselaar, 1994; Campbell et al., 1996), which lead to an increasing interest in the use of medicinal plants as alternative management of type 2 diabetes mellitus.

*Orthosiphon stamineus* Benth (Lamiaceae) is a popular medicinal plant in Southeast Asia. It is widely used for the treatments of many diseases, especially those affecting the urinary tract, diabetes mellitus, hypertension, rheumatism, tonsillitis and menstrual disorder (Awale et al., 2003a,b). The methanolic extracts of this plant have shown the inhibitory activity on nitric oxide production in macrophage like cells (Awale et al., 2003a,b). Moreover, the major chemicals of *Orthosiphon stamineus*, i.e., polymethoxylated flavonoids and caffeic acid derivatives, exert the diuretic and uricosuric actions in rats (Olah et al., 2003). To the best of our knowledge, there has been no report regarding hypoglycemic effect of *Orthosiphon stamineus* aqueous extract in normal and diabetic rats. The present study was to evaluate the anti-hyperglycemic activity of aqueous extract of *Orthosiphon stamineus* in normal and STZ-induced

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RESEARCH ARTICLE

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# Comprehensive chemical and metabolic profiling of anti-hyperglycemic active fraction from Clerodendranthi Spicati Herba

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Extensive pharmacological research has demonstrated that Clerodendranthi Spicati Herba has an obvious anti-hyperglycemic effect via α-glucosidase inhibitory activity. However, the anti-hyperglycemic active fraction and its metabolic behavior in vivo have not been elaborated clearly. In this study, ultra-high-performance liquid chromatography coupled to quadrupole time of flight tandem mass spectrometry with data filtering strategy, including mass defect screening, diagnostic product ions and neutral loss identification, was established for chemical and metabolic profiling of anti-hyperglycemic active fraction from Clerodendranthi Spicati Herba. A total of 28 methoxylated flavonoids and 61 diterpenoids were rapidly identified. Four main known methoxylated flavonoids were purified and unambiguously identified by nuclear magnetic resonance analysis. Thirty-one absorbed diterpenoids, 12 absorbed methoxylated flavonoids, and 56 methoxylated flavonoids metabolites were identified in rat plasma, urine, bile, and feces after oral administration of anti-hyperglycemic active fraction. The methoxylated flavonoids were predominantly metabolized by demethylation, sulfation, and glucuronidation. Glucuronidation metabolites found in bile and urine after demethylation were dominant metabolites. Diterpenoids were absorbed into the blood mainly in the form of prototypes and excreted through bile and urine. These results indicated that methoxylated flavonoids and diterpenoids were responsible for α-glucosidase inhibitory activity, which might provide novel drug candidates for the management of diabetes mellitus.

**KEYWORDS**
anti-hyperglycemic effect, Clerodendranthi Spicati Herba, data filtering strategy, diterpenoids, methoxylated flavonoids

## 1 | INTRODUCTION

Clerodendranthi Spicati Herba (CSH), the dried aerial part of *Clerodendranthus spicatus* (Thunb.) C.Y. Wu, was traditionally used as an edible and medicinal material in China, Malaysia, Vietnam, Indonesia, and other Southeast Asian countries, which is commonly applied to treat diabetes,

**Article Related Abbreviations:** AUC, area under the curve; CSH, Clerodendranthi Spicati Herba; DAD, diode array detector; DPIs, diagnostic product ions; EEF, ethanol elution fraction; MFs, methoxylated flavonoids; NL, neutral loss; RDA, Retro-Diels-Alder

  

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Original Article

# Clinical Characteristics for the Relationship between Type-2 Diabetes Mellitus and Cognitive Impairment: A Cross-Sectional Study

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**ABSTRACT:** We explored the potential differences in cognitive status, lipid and glucose metabolism, ApoEε4 alleles and imaging between diabetic and non-diabetic subjects. 83 subjects with normal cognitive function and 114 mild cognitive impaired patients were divided into four groups by history of diabetes. General demographics was collected from all participants followed by MRI scan, biochemical examinations and a series of neuropsychological tests. Student's t test, multiple regressions and one-way ANOVA were applied to investigate the differences between groups. Comparing diabetic patients with non-diabetic subjects in the mild cognitive impaired group, we found several decreased items in recall of three words in MMSE ($p$=0.020), AVLT and SCWT ($p<0.050$). The multiple linear regression revealed that two-hour glucose level (B= -0.255, $p<0.001$) and fasting C-peptide (B= -0.466, $p$=0.001) had negative effects on the score of MMSE. In addition, diabetic patients treated with insulin and other diabetes medication performed better in part of the AVLT ($p<0.050$) compared to patients with insulin treatment or oral antidiabetic medication only. Patients with metformin medication had a better memory outcome compared to patients with sulphonylurea medication in the AVLT long delay free recall ($p$ =0.010). These findings show that patients of mild cognitive impairment with diabetes mellitus have a worse outcome in attention, information processing speed and memory compared to non-diabetic patients. Higher two-hour glucose level and C-peptide level may be risk factors for severe cognitive impairment in type-2 diabetes mellitus patients. The results of this study also suggest that medication may have effects on cognitive function.

***Key words:*** Type-2 diabetes mellitus, mild cognitive impairment, C-peptide, blood glucose

The incidence of type-2 diabetes mellitus (T2DM) in China has dramatically increased in the last decade. Besides the well-known connection between T2DM and peripheral nervous system disease, the diabetes-induced lesions in the central nervous system (CNS), such as cerebrovascular disease and cognitive dysfunction, are receiving increased attention. Several epidemiological studies have found that T2DM is an independent risk factor for both Alzheimer's disease (AD) and vascular dementia (VaD) [1, 2]. In addition, further research has shown that T2DM may also exercise influence on the prevalence of mild cognitive impairment (MCI), which is considered a pre-clinical stage of dementia. In Luchsinger and colleagues' study [3], the results indicated that diabetes mellitus is related to a relatively higher risk for all causes of MCI (about 1.5 fold). In another recent meta-analysis report, diabetes was shown to have higher risk for any dementia and MCI (1.46 for AD, 2.48 for VaD and 1.21 for MCI) [4].

The present studies have confirmed that T2DM is a robust risk factor for cognitive dysfunction. However, the precise mechanisms remain to be elucidated [5]. A wide range of metabolic and vascular disturbances have been proposed to explain the underlying mechanisms of

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