J Physiol Biochem (2014) 70:675–684
DOI 10.1007/s13105-014-0337-0

ORIGINAL PAPER

Cytoprotective and anti-diabetic effects of *Derris reticulata* aqueous extract

Pakarang Kumkrai · Sirilak Kamonwannasit · Nuannoi Chudapongse

Received: 4 December 2013 / Accepted: 12 May 2014 / Published online: 22 May 2014

Abstract The current study was aimed to investigate pancreatic protective and anti-diabetic activities of the aqueous extract of *Derris reticulata* stem. First, we evaluated a cytoprotective potential of *D. reticulata* extract on alloxan-induced damage in vitro. Treatment with *D. reticulata* extract at the doses of 250 and 500 μg/ml significantly increased cell viability of the pancreatic β-cell line RINm5F after exposure of alloxan. The anti-hyperglycemic activity of *D. reticulata* extract was further studied in alloxan-induced diabetic rats. A significant reduction in blood glucose level along with an increase in body weight was observed in diabetic rats treated with *D. reticulata* extract at 250 mg/kg body weight for 15 days. Serum aspartate transaminase and alanine transaminase levels were also significantly decreased compared to diabetic control rats. In accordance with in vitro cytoprotective effect, histopathological examination revealed that pancreatic islet cells of the extract-treated diabetic rat were less damage than those of the untreated diabetic group. In order to find another possible mechanism of action underling hypoglycemic activity, the effect on glucose absorption was examined using everted sac jejunum. The results showed that *D. reticulata* extract suppressed glucose absorption from small intestine. To corroborate safety use of *D. reticulata* extract, acute oral toxicity was also conducted in rats. Our results showed that none of the tested doses (250, 500, 1,000, and 2,000 mg/kg) induced signs of toxicity or mortality after administration of the extract. The results suggested that *D. reticulata* extract possess anti-diabetic activity, which resulting from its pancreatic cytoprotective effect and inhibition of intestinal glucose absorption.

Keywords *Derris reticulata* · Anti-hyperglycemic · Alloxan · Diabetic rats · Glucose absorption · RINm5F cells

P. Kumkrai · S. Kamonwannasit · N. Chudapongse (✉)
School of Pharmacology, Institute of Science, Suranaree University of Technology,
Nakhon Ratchasima 30000, Thailand
e-mail: nuannoi@sut.ac.th

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from reduction of insulin secretion and/or insulin resistance. The estimated number of people suffering from diabetes (aged 20–79 years) affected 285 million in the year of 2010 and will increase to 439 million in the year 2030. In developed countries, the trend is that the prevalence of people with diabetes is aged over 60 years. In contrast, diabetes in developing countries is widely occurring in working age population (aged 40–60 years). This problem is relevant to socioeconomic growth and urbanization, resulting in an increase amount of patients with diabetes worldwide [26]. Chronic hyperglycemia is involved in several complications such as retinopathy, nephropathy, neuropathy, and cardiovascular disease [2]. Thus, blood glucose level control can reduce the progress of disease,

morbidity, and mortality [30]. In this sense, in addition to modern drugs available in markets, several medicinal plants have also been used as alternative medicines for diabetes treatment. A search for new traditional anti-diabetic medicines has been recommended by the World Health Organization experts [34].

Derris reticulata Craib., commonly known in Thai as cha-em-nuea, is a climbing plant which belongs to Leguminosae family. Several plants of the genus *Derris* have been reported to possess a variety of biological activities. For example, *Derris trifoliata* is used for treatment of rheumatism, dysmenorrhea, and asthma [8] and *Derris scandens* is used to treat arthritis patients [15]. In the case of *D. reticulata*, it has been used as an expectorant to relieve cough [33]. Moreover, all of them have been reported to possess antimicrobial activities [5, 12, 28]. It has been shown that the extract from *D. scandens* stem significantly reduces myeloperoxide release and inhibits the generation of leukotriene B_4 [15], which supports its traditional use as an anti-inflammatory agent. Similarly, the ethanolic extract from stem of *D. reticulata* has been demonstrated to exert anti-inflammatory activity by interfering with nitric oxide production of macrophage [33]. *Derris indica* and *D. scandens* exhibits free radical scavenging activity [22, 23]. It has been reported that *D. reticulata* is compose of flavonoids similar to several plants in the genus *Derris* [29]. In this sense, diabetes has been implicated in an increase formation of free radical [32] while it is widely known that flavonoid compounds can act as antioxidants. Taken together, it is possible that *D. reticulata* has antioxidant potential and could be beneficial for treatment of diabetes. Not surprisingly, it has been employed as alternative diabetes treatment by local medicinal plant practitioners in some parts of Thailand. However, to our best knowledge, scientific evidence supporting anti-hyperglycemic activity of *D. reticulata* has never been documented.

First, we examined an in vitro protective effect of *D. reticulata* extract using the insulin-secreting cell line RINm5F. Second, anti-hyperglycemic activity was evaluated in alloxan-induced diabetic rats and histopathological study was performed to confirm the protective effect of the extract in vivo. Third, we investigated another possible mechanism of the anti-hyperglycemic effect on glucose absorption in vitro. Finally, to assure safety use of *D. reticulata* extract, acute oral toxicity in rats was also conducted in this study.

Materials and methods

Collection of plant material and extraction

The plants were collected from Prachinburi province, Thailand. A voucher specimen (Pharm-Chu-006) was deposited at School of Pharmacology, Suranaree University of Technology (SUT). The stems were cut into small pieces and dried at 50 °C in a hot air oven. *D. reticulata* extract was prepared by boiling 100 g of dried plants in 500 ml of distilled water for 10 min. This process was repeated twice. The aqueous extract of *D. reticulata* was filtered through cotton gauze and then centrifuged at 2,500×*g* for 10 min. Supernatant was collected and lyophilized. The dried extract (yield 16.73 %, *w*/*w*) was kept at −20 °C until used.

Cells and culture conditions

RINm5F, *Rattus norvegicus* (rat) cell line was obtained from the American Type Culture Collection (ATCC, Manassas, USA). RINm5F cells were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 1 % antibiotic-antimycotic solution and incubated at 37 °C in a humidified atmosphere containing 5 % CO_2.

In vitro cytoprotective study

To investigate cytoprotective effect of *D. reticulata* extract on alloxan-induced RINm5F cell damage, the experiment was carried out according to the procedure described earlier [18] with minor modifications (different incubation periods).

Effect of D. reticulata extract on the viability of RINm5F cells

The effects of *D. reticulata* extract on the viability of RINm5F cells were first tested to find the range of optimal concentrations in the cytoprotective experiment. RINm5F cells were seeded in 96-well plates at the concentration of 2×10^5 cells/well. After allowed to attach overnight, cells were treated with a various concentrations of *D. reticulata* extract at the doses of 0–3,500 μg/ml for 24 h. At the end of incubation, cell viability was determined by MTT assay as follow. After treatment, the medium was removed and then 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT, Life Technologies, Carlsbad, USA) was added. Cells were then incubated in the dark at 37 °C for an additional 4 h. After the incubation, formazan crystals were formed. The MTT solution was removed and the formazan crystals were dissolved in 50 μl of DMSO. Absorbance was read at 570 nm using a microplate reader. Absorbance of the reaction solution without cells was set as background. The background (absorbance of the reaction solution without cells) was subtracted from the sample value before data analysis. The absorbance of untreated cells was considered as 100 %.

Effect of alloxan on the viability of RINm5F cells

In order to find the suitable concentration of alloxan for induction of 50 % cell damage, RINm5F cells with density of 2×10^5 cells/well were seeded in 96-well plates and then treated with alloxan at a range of concentrations 0–15 mM (dissolved in 50 mM citrated buffer pH 3.0). After 1 h of incubation, the medium was removed and then cells were additional incubated for 23 h. At the end of experiment, cell viability was assessed by MTT assay as described in the previous section.

Determination of cell viability on alloxan-induced cellular damage

RINm5F cells (2×10^5) were incubated with alloxan at 9 mM (which causing about 50 % of cell death). After 1 h of incubation, the medium containing alloxan was removed. After that, cells were treated with *D. reticulata* extract for further 23 h. At the end of experiment, cell viability was assessed by MTT assay as described earlier.

Animals

Male and female Wistar rats used in this study were obtained from Laboratory Animal Center, SUT. Animals were acclimatized for 7 days prior to the experiments. The rats were housed in polypropylene cages, with free access to normal diet and water ad libitum. The rats were maintained at room temperature (25±0.5 °C), relative humidity 45–50 % and at 12-h light/dark cycle. All procedures in this study were approved and conducted according to guidelines of the Institutional Animal Care and Use Committee, SUT. All efforts were made to minimize the number of rats used and their suffering.

Investigation of anti-hyperglycemic activity

Induction of diabetes

Diabetic rats were induced by intraperitoneal injection of alloxan monohydrate in fasted male rats at the dose 150 mg/kg [20]. Alloxan was freshly dissolved in 0.85 % (*w*/*v*) NaCl. Diabetic rats were orally given 5 % glucose solution overnight after alloxan injection to prevent hypoglycemia. On the third day, rats with fasting blood glucose level higher than 250 mg/dl were considered as diabetic rats and used in the experiment. Animals were divided into four groups, and each group included five rats as follows: group I (NC), normal control rats received sterile water; group II (DM), diabetic control rats received sterile water, group III (DM+ extract), diabetic rats treated with *D. reticulata* extract at 250 mg/kg; and group IV (DM+glib), diabetic rats treated with glibenclamide tablets 20 mg/kg. The extract and standard drug were administered once a day for 15 days. The freshly prepared solutions were orally administered to animals by gastric intubation with force feeding needle.

Sample collection

Measurement of body weight and blood glucose level was done on the fifth, tenth, and 15th day of the treatment. Blood samples were collected from tail vein for determination of fasting blood glucose level using Glucometer Accu-Chek Performa glucose test strips (Roche Diagnostics, Indianapolis, USA). At the end of experiment, blood samples were collected via cardiac puncture for biochemical analyses. Then, the animals were sacrificed by cervical dislocation. Pancreas was removed and rinsed in 0.85 % (w/v) NaCl. Tissue samples were fixed with 10 % neutral buffered formaldehyde for histopathological examinations.

Biochemical parameters

For biochemical parameters analysis, blood sample without anticoagulant was centrifuged at 3,000×*g* for 5 min to obtain serum and then stored at −20 °C until analysis. Aspartate transaminase (AST) and alanine

transaminase (ALT) activities were determined by A15 Random Access Analyzer (Biosystems S.A., Spain). The enzyme activities indicate the leakage of these enzymes from liver to blood stream [6].

Histopathological examinations

Tissue samples of pancreas were fixed with 10 % neutral buffered formaldehyde and dehydrated by serial ethanol solutions (70, 95, and 100 %, respectively). The tissues were embedded in paraffin and sectioned into 5-μm thickness. The sections were stained with hematoxylin and eosin. The photomicrograph of each tissue section was observed under microscope (model CX31, Olympus, Tokyo, Japan).

Effect of D. reticulata extract on inhibition of glucose absorption

Inhibition of glucose absorption was investigated in rat intestine using a previously described method [14]. Rat jejunum was removed immediately after cervical dislocation and immersed in Kreb-Henseleit solution continuously aerated with carbogen. The jejunum was cut into several pieces and everted with glass rod. Each everted jejunum was tied with a cotton thread at one end. Kreb-Henseleit solution with 140 mg/dl glucose was filled into the everted jejunum, and then the other end of jejunum was tied. Each jejunum sacs was incubated in the following solutions: group I, Kreb-Henseleit with glucose 140 mg/dl; group II, Kreb-Henseleit with glucose 140 mg/dl mixed with sodium fluoride (NaF) 0.2 M; groups III–V, Kreb-Henseleit with glucose 140 mg/ml mixed with *D. reticulata* extract at 0.25, 0.5, and 2.5 mg/ml, respectively. The incubation flasks were shaken at 90 oscillation/min, 37 °C for 30 min. After the incubation, the sacs were cut and the concentration of glucose in the sac was examined using peroxidase-glucose oxidase (PGO) enzyme commercial kit (Sigma-Aldrich, St. Louis, USA).

Acute toxicity study

Acute oral toxicity was performed based on the guidelines of the OECD (2001) [17]. After acclimatization, rats were randomly assigned to five groups (four males and four females each). *D. reticulata* extract at the doses of 250, 500, 1,000, and 2,000 mg/kg was administered to rats. The behavior of rats was continuously monitored for 1 h and then once daily for 14 days. The toxicity signs and symptoms were examined as described previously [3].

Statistical analysis

Data are expressed as mean±SEM. Comparisons among different groups were performed by analysis of variance (ANOVA) followed by Student-Newman-Keuls test. *P* values less than 0.05 were set as the level of significance.

Results

Effect of *D. reticulata* extract on alloxan-induced RINm5F cell damage

As shown in Fig. 1a, after treated with *D. reticulata* extract at the concentrations higher than 500 μg/ml for 24 h, the viability of RINm5F cells were significantly decreased compared to control. *D. reticulata* extract at the concentrations of 50–500 μg/ml which did not significantly reduce cell viability were selected for further study. Figure 1b showed the results from cytotoxicity study of alloxan. The half maximal inhibitory concentration (IC_{50}) of alloxan on cell viability was found at 9 mM which was used for studying the cytoprotective effect of the extract. Post-treatment with *D. reticulata* extract was found to have significant protective action against alloxan-induced RINm5F cells damage in dose-dependent fashion as shown in Fig. 1c.

Anti-hyperglycemic activity of the extract

Effect of D. reticulata extract on body weight and fasting blood glucose levels

The body weights were found to increase normally in control rats. Diabetic control rats induced by alloxan at the dose of 150 mg/kg had significantly weight loss, whereas treatments with *D. reticulata* extract (250 mg/kg) and glibenclamide (20 mg/kg) significantly

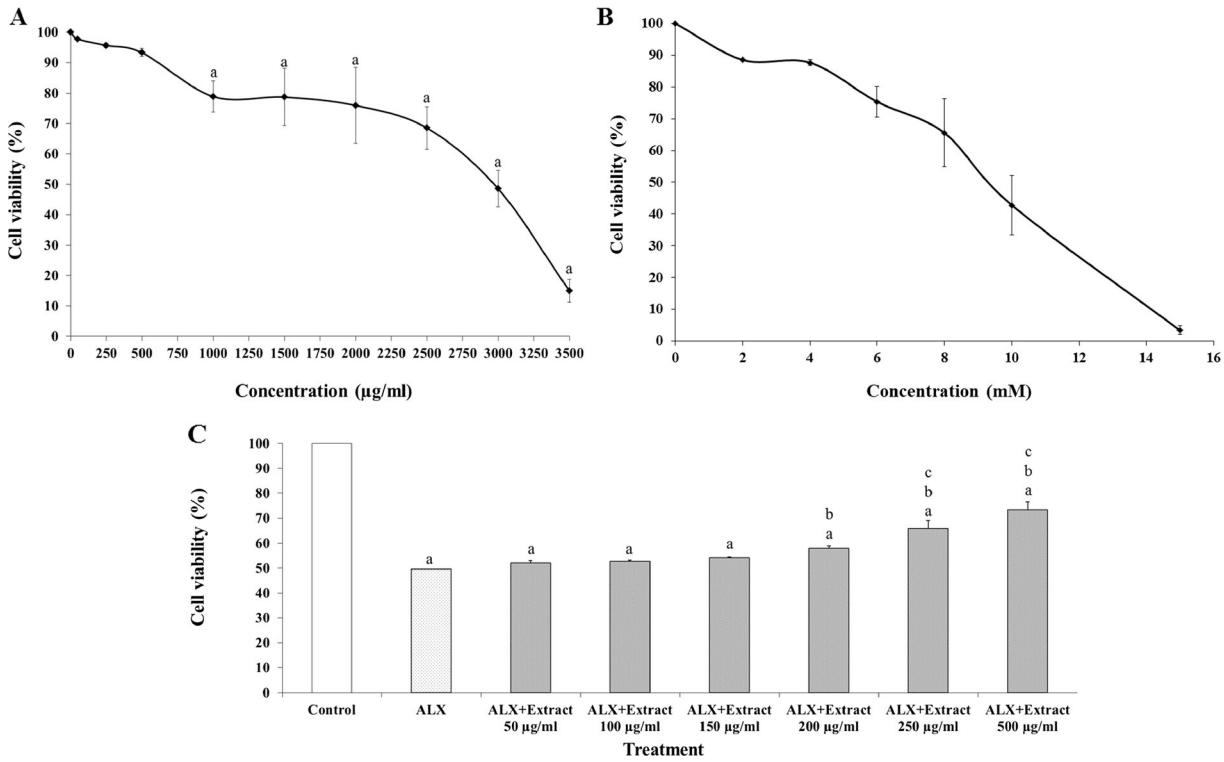

Fig. 1 Cytoprotective effect of *D. reticulata* extract. **a**–**b** %Viability of RINm5F cells after treatment with various concentrations of the extract and alloxan, respectively. The concentrations of the extract higher than 500 µg/ml significantly reduced %cell viability compared to control. The calculated IC_{50} of the extract and alloxan were 2,896 µg/ml and 9 mM, respectively. **c** The protective effect of the extract on %cell viability after exposure to alloxan at 9 mM. Values are expressed as mean±SEM. (n=3). $^{a}p<0.05$ statistically significant difference from control. $^{b}p<0.05$ statistically significant difference from alloxan. $^{c}p<0.05$ statistically significant difference from the lower doses of each dose

improved the body weight as shown in Table 1. Intraperitoneal injection of alloxan caused elevation of blood glucose however treatment with *D. reticulata* extract and glibenclamide resulted in a reduction of blood glucose levels when compare with diabetic control rats within 15 days.

Effect of D. reticulata extracts on biochemical parameters

The levels of liver enzymes (AST and ALT) in normal and experimental rats are shown in Table 2. As expected, the levels of AST and ALT were markedly increased in alloxan-induced diabetic rats. However, these hepatic enzymes in serum decreased significantly by the treatment with *D. reticulata* extract, similar to the treatment with glibenclamide as positive control.

Protective effect of D. reticulata extract on pancreatic islets

Histopathological examinations were used to evaluate the protective effect of *D. reticulata* extract in vivo. Severe pancreatic tissue damages were observed in alloxan-induced diabetic control rats (Fig. 2b). In addition, the size of pancreatic islets were decreased and shrunk when compared to that of normal rats (Fig. 2a). As seen in Fig.2c–d, the pancreatic islets injury were restored after treated with *D. reticulata* extract and glibenclamide, respectively.

Inhibitory effect of D. reticulata extract on glucose absorption

Inhibition of intestinal glucose absorption by *D. reticulata* extract was examined by everted intestinal sacs method. As presented in Table 3, glucose

Table 1 Effect of *D. reticulata* extract on body weight and fasting blood glucose levels

Treatment	Body weight (g)		Blood glucose levels (mg/dl)			
	Initial	Final	0 day	5 days	10 days	15 days
NC	234.00±5.70	274.00±10.37	82.80±2.79	79.00±3.48	82.80±1.51	81.20±2.27
DM	230.00±3.53	190.00±10.00*	335.80±3.13	346.20±3.41*	355.40±4.08*	406.60±12.90*
DM+extract, 250 mg/kg	230.00±9.35	222.00±12.45*, **	332.60± 12.94	275.20±14.53*, **	240.40±10.01*, **	172.80±6.57*, **
DM+glib, 20 mg/kg	225.00±7.50	230.00±6.12*, **	336.00±7.17	263.00±18.07*, **	220.80±17.84*, **	126.20±5.95*, **

Values are expressed as mean±SEM (n=5/group)

NC normal control rats received sterile water, *DM* diabetic control rats received sterile water, *DM+extract* diabetic rats treated with the extract, *DM+glib* diabetic rats treated with glibenclamide

*p<0.05, statistically significant difference from normal control

**p<0.05, statistically significant difference from diabetic control

concentrations inside the sacs incubated with the extract and NaF (positive control) significant decreased compared to negative control (p<0.05). The results showed that *D. reticulata* extract at the doses of 0.25, 0.5, and 2.5 mg/ml suppressed glucose absorption from intestine. The extract exerted this inhibitory effect in dose-dependent manner.

Acute toxicity

Oral administration of *D. reticulata* extract at the doses of 250, 500, 1,000, and 2,000 mg/kg did not induce abnormal behavior in rats of both sexes. Normal body weight gains were observed in male and female rats as shown in Table 4. There were no animal deaths during 14 days after administration of the extract. Therefore, the lethal dose of *D. reticulata* extract was higher than 2,000 mg/kg. No gross abnormalities of internal organs were observed in animal treated with *D. reticulata* extract.

Table 2 Effect of *D. reticulata* extract on biochemical parameters

Treatment	Liver enzymes	
	AST (U/l)	ALT (U/l)
NC	75.00±1.77	28.60±1.48
DM	309.00±73.20*	65.60±24.45*
DM+extract, 250 mg/kg	119.40±4.08*, **	37.60±4.62*, **
DM+glib, 20 mg/kg	122.80±5.04*, **	37.40±5.04*, **

Values are expressed as mean±SEM (n=5/group)

NC normal control rats received sterile water, *DM* diabetic control rats received sterile water, *DM+extract* diabetic rats treated with the extract, *DM+glib* diabetic rats treated with glibenclamide

*p<0.05, statistically significant difference from normal control

**p<0.05. statistically significant difference from diabetic control

Discussion

It is widely accepted that plants are useful sources of remedies for many diseases. Phenolic compounds in plants have exhibited health protective effects in many ailments such as diabetes and hypertension [21]. *D. reticulata* has been traditionally used for the treatment of diabetic patients in some areas in Thailand. However, there is no scientific evidence available supporting the anti-diabetic activity of this plant. In this study, at first we evaluated a cytoprotective potential of *D. reticulata* extract on alloxan-induced cell death in the pancreatic β-cell line RINm5F and then examined anti-hyperglycemic activity in alloxan-induced diabetic rats. An injection of alloxan results in a sustainable hyperglycemia, which is a critical feature of alloxan-induced diabetic rats [1]. Alloxan selectively destroys the pancreatic β-cells leading to a decrease of insulin secretion [16]. The insulin reduction causes poor glucose utilization in experimental animals, resulting in an increase catabolism, protein depletion, muscle wasting, and loss of body weights [11, 25]. It was found that *D. reticulata* extract at the doses of 250 and 500 μg/ml increased cell viability after exposure of alloxan from about 50 % to 66 and 73 % of control, respectively. It is known that

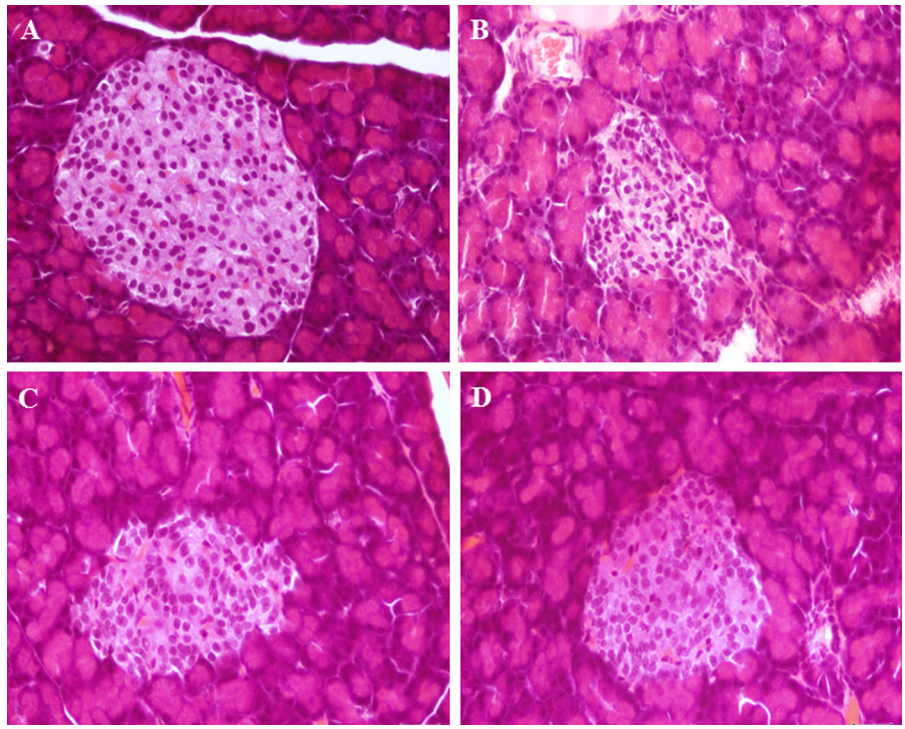

Fig. 2 Photomicrograph of pancreatic islets of normal and diabetic rats. Hematoxylin and eosin staining (×400); **a** normal control rats, **b** diabetic control rats receiving steriled water, **c** diabetic rats treated with the extract (250 mg/kg), and **d** diabetic rats treated with glibenclamide (20 mg/kg)

alloxan causes cell death by the generation of reactive oxygen species [13, 31]. Several lines of evidence have suggested that anti-hyperglycemic effects of plants are associated with their antioxidant activity, which is likely due to the presence of phenolic phytochemicals in the plant extracts [10, 19, 24]. It is widely known that phenolic compounds in plants comprise several groups of phytochemicals such as tannin, terpenoids, and flavonoids. *Solanum torvum* Swartz extract containing phenolic compounds, such as rutin, caffeic acid, gallic acid, and catechin, exhibits hypoglycemic activity and are known for their ability to regenerate β-cells from damage induced by streptozotocin [7]. Flavonoids found in *Potentilla discolor* extract also have protective

Table 3 Inhibitory effect of *D. reticulata* extract on glucose absorption

Treatment	Glucose concentration inside the sacs (mg/dl)	%Inhibition
Control	306.84±4.89	0.00±0.00
Extract, 0.25 mg/ml	283.47±6.84*	7.65±0.88*
Extract, 0.5 mg/ml	269.65±3.73*, **	12.1±1.01*, **
Extract, 2.5 mg/ml	225.63±7.11*, **	26.4±2.26*, **
NaF, 0.2 M	188.02±2.38*	38.7±0.71*

Values are expressed as mean±SEM (n=5)

*p<0.05, statistically significant difference from control

**p<0.05, statistically significant difference from the lower doses

Table 4 Body weight changes and toxic signs of rats given a single dose of the aqueous extract of *D. reticulata*

Treatment	Body weight (g)			Mortality	Symptom of toxicity	Gross pathology
	0 day	7 days	14 days			
Male						
Control	220.00±9.13	237.50±8.54	260.00±14.72	0	None	Normal
Extract, 250 mg/kg	220.00±2.50	230.00±4.08	247.50±2.50	0	None	Normal
Extract, 500 mg/kg	222.50±11.75	235.00±10.21	255.00±13.98	0	None	Normal
Extract, 1,000 mg/kg	232.50±7.50	262.50±10.31	270.00±10.80	0	None	Normal
Extract, 2,000 mg/kg	227.50±7.50	260.00±9.31	270.00±12.91	0	None	Normal
Female						
Control	180.00±5.77	192.50±7.50	202.50±6.29	0	None	Normal
Extract, 250 mg/kg	187.50±4.79	200.00±7.07	202.50±8.54	0	None	Normal
Extract, 500 mg/kg	177.50±2.50	192.50±7.50	192.50±7.50	0	None	Normal
Extract, 1,000 mg/kg	187.50±7.50	200.00±8.16	210.00±7.07	0	None	Normal
Extract, 2,000 mg/kg	190.00±5.77	202.50±2.50	210.00±4.08	0	None	Normal

Values are expressed as mean±SEM (n=4/group)

There was no significant difference between control and the treated groups (p>0.05)

effects on β-cells in streptozotocin-induced diabetic rats [35]. Some flavonoids, which are found in other *Derris* plants, have been identified and isolated from *D. reticulata* Craib [29]. The extracts from both *D. scandens* and *D. reticulata* has been reported to possess anti-inflammatory activity [15, 33]. Taken together, the underlying mechanism of cytoprotective action of *D. reticulata* extract could possibly be related to the inhibition of oxidative stress and anti-inflammatory activities. However, experimental evidence supporting this speculation is needed.

Normally, plants contain natural phenolic compounds which can produce antioxidant properties in vitro, though it is not guarantee that those plant extracts would exert useful therapeutic actions in vivo for several reasons. For example, the active ingredients may not be absorbed from the site of administration or enzymatic degradation may occur before reaching the target organ. However, the data from this study showed that *D. reticulata* extract produced anti-diabetic activity against alloxan induction in vivo. The extract significantly increased the body weights of diabetic rats compared to the diabetic control group. In accordance with the results on body weights, diabetic rats treated with *D. reticulata* extract showed significant decreases in blood glucose level similar to glibenclamide-treated group. The microscopic photograph from pancreatic hematoxylin/eosin staining demonstrated a destruction of the pancreatic islet cells, such as shrink and irregular shape, in diabetic control rats. The islet cells of the diabetic rats treated with *D. reticulata* extract provides the evidence of less degeneration. An increase in the levels of hepatic enzymes, such as AST, ALT, glutamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transferase (GPT), has been established in diabetic rats induced by toxic chemicals, including alloxan and streptozotocin [6, 9]. In this study, alloxan-induced increase of AST and ALT were decreased in rats administrated with *D. reticulata* extract compared to diabetic control group. In addition to pancreas, the result suggested that the extract also exerted hepatoprotective activity.

An impact of postprandial hyperglycemia risk on diabetic complication progress has been reported [4]. The reduction of postprandial hyperglycemia has been approached by suppression of carbohydrate absorption from gastrointestinal tract [27]. The effect of *D. reticulata* extract on glucose absorption was examined using everted sac of jejunum. The data showed that incubation of *D. reticulata* extract at the doses of 0.25, 0.5, and 2.5 mg/ml inhibited glucose absorption from

small intestine, which implicating a possible mechanism underlying its anti-hyperglycemic effect. Moreover, this result suggested that the extract could be useful for enhancing the glycemic control on postprandial hyperglycemia.

A number of chemicals or plant extracts have been shown to possess potent activities both in vitro and in vivo; however, some of them may not be used as therapeutic drugs due to high toxicity. To assure the potential of this extract for clinical use, acute toxicity study was conducted. The result showed that single dose oral administration of *D. reticulata* extract at 250, 500, 1,000, and 2,000 mg/kg to male and female rats did not cause mortality or produce any remarkable toxic signs and adverse effects on gross histopathology of rats. The results indicated that LD_{50} of the extract was greater than 2,000 mg/kg. Thus, the aqueous extract of *D. reticulata* can be classified as category 5 (low or no toxicity) in accordance with Globally Harmonized Classification System of OECD (2001) [17].

Conclusion

The data obtained from the present study provide valuable information that *D. reticulata* extract exerts an anti-diabetic activity via cytoprotective effect on pancreatic β-cells and inhibitory action on glucose absorption with a relatively wide margin of safety. However, more efforts are still needed for the isolation, characterization and pharmacological evaluation of the active compound(s) in *D. reticulata* extract.

Acknowledgments This study was financially supported by SUT Research and Development Fund, and the National Research Council of Thailand. We thank Dr. Paul J. Grote for plant botanical classification verification.

References

1. Adeneye AA, Adeyemi OO (2009) Further evaluation of antihyperglycaemic activity of *Hunteria umbellata* (K. Schum) Hallier f. seed extract in experimental diabetes. J Ethnopharmacol 126:238–243
2. American Diabetes Association (2008) Diagnosis and classification of diabetes mellitus. Diabetes Care 31:S55–S60
3. Carvalho ALN, Annoni R, Silva PRP, Borelli P, Fock RA, Trevisan MTS et al (2011) Acute, subacute toxicity and mutagenic effects of anacardic acids from cashew (*Anacardium occidentale* Linn.) in mice. J Ethnopharmacol 135:730–736
4. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B et al (2002) Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation 106:1211–1218
5. Chivapat S, Chavalittumrong P, Attawish A, Soonthornchareonnon N (2009) Toxicity study of lupinifolin from stem of *Derris reticulata* Craib. J Thai Tradit Altern Med 7:146–155
6. Eliza J, Daisy P, Ignacimuthu S, Duraipandiyan V (2009) Antidiabetic and antilipidemic effect of eremanthin from *Costus speciosus* (Koen.)Sm., in STZ-induced diabetic rats. Chem Biol Interact 182:67–72
7. Gandhi GR, Ignacimuthu S, Paulraj MG (2011) *Solanum torvum* Swartz. fruit containing phenolic compounds shows antidiabetic and antioxidant effects in streptozotocin induced diabetic rats. Food Chem Toxicol 49:2725–2733
8. Jiang C, Liu S, He W, Luo X, Zhang S, Xiao Z et al (2012) A new prenylated flavanone from *Derris trifoliata* Lour. Molecules 17:657–663
9. Ju JB, Kim JS, Choi CW, Lee HK, Oh TK, Kim SC (2008) Comparison between ethanolic and aqueous extracts from Chinese juniper berries for hypoglycaemic and hypolipidemic effects in alloxan-induced diabetic rats. J Ethnopharmacol 115:110–115
10. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK (2006) Antidiabetic agents from medicinal plants. Curr Med Chem 13:1203–1218
11. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S et al (2010) Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. Food Chem Toxicol 48:1078–1084
12. Khan MR, Omoloso AD, Barewai Y (2006) Antimicrobial activity of the *Derris elliptica*, *Derris indica* and *Derris trifoliata* extractives. Fitoterapia 77:327–330
13. Kim HR, Rho HW, Park BH, Park JW, Kim JS, Kim UH et al (1994) Role of Ca^{2+} in alloxan-induced pancreatic β-cell damage. Biochim Biophys Acta 1227:87–91
14. Krisanapun C, Peungvicha P, Temsiririrkkul R, Wongkrajang Y (2009) Aqueous extract of *Abutilon indicum* Sweet inhibits glucose absorption and stimulates insulin secretion in rodents. Nutr Res 29:579–587
15. Laupattarakasem P, Houghton PJ, Hoult JRS, Itharat A (2003) An evaluation of the activity related to inflammation of four plants used in Thailand to treat arthritis. J Ethnopharmacol 85:207–215
16. Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51:216–226
17. OECD (2001) Acute oral toxicity - acute toxic class method. In: OECD guideline for testing of chemicals No 423. Available from http://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en Accessed on 10 January 2012

18. Ramkumar KM, Lee AS, Krishnamurthi K, Devi SS, Chakrabarti T, Kang KP et al (2009) *Gymnema montanum* H. protects against alloxan-induced oxidative stress and apoptosis in pancreatic β-cells. Cell Physiol Biochem 24:429–440
19. Ramkumar KM, Manjula C, Sankar L, Suriyanarayanan S, Rajaguru P (2009) Potential in vitro antioxidant and protective effects of *Gymnema montanum* H. on alloxan-induced oxidative damage in pancreatic β-cells, HIT-T15. Food Chem Toxicol 47:2246–2256
20. Ramkumar KM, Vijayakumar RS, Ponmanickam P, Velayuthaprabhu S, Archunan G, Rajaguru P (2008) Antihyperlipidaemic effect of *Gymnema montanum*: a study on lipid profile and fatty acid composition in experimental diabetes. Basic Clin Pharmacol 103:538–545
21. Randhir R, Kwon YI, Shetty K (2008) Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings. Innov Food Sci Emerg 9:355–364
22. Ranga Rao R, Tiwari AK, Prabhakar Reddy P, Suresh Babu K, Ali AZ, Madhusudana K et al (2009) New furanoflavanoids, intestinal α-glucosidase inhibitory and free-radical (DPPH) scavenging, activity from antihyperglycemic root extract of *Derris indica* (Lam.). Bioorg Med Chem 17:5170–5175
23. Rao SA, Srinivas PV, Tiwari AK, Vanka UMS, Rao RVS, Dasari KR et al (2007) Isolation, characterization and chemobiological quantification of α-glucosidase enzyme inhibitory and free radical scavenging constituents from *Derris scandens* Benth. J Chromatogr B 855:166–172
24. Sabu MC, Kuttan R (2002) Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol 81:155–160
25. Shabeer J, Srivastava RS, Singh SK (2009) Antidiabetic and antioxidant effect of various fractions of *Phyllanthus simplex* in alloxan diabetic rats. J Ethnopharmacol 124:34–38
26. Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87:4–14
27. Si MM, Lou JS, Zhou CX, Shen JN, Wu HH, Yang B et al (2010) Insulin releasing and alpha-glucosidase inhibitory activity of ethyl acetate fraction of *Acorus calamus in vitro* and *in vivo*. J Ethnopharmacol 128: 154–159
28. Sittiwet C, Puangpronpitag D (2009) Antimicrobial properties of *Derris scandens* aqueous extract. J Biol Sci 9:607–611
29. Songsak T (1995) Phytochemistry of stem bark and pharmacognostic specification of *Derris reticulata* Craib. In: Master's Thesis Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, pp 267
30. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA et al (2000) Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321:405–412
31. Szkudelski T (2001) The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 50:537–546
32. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 39:44–84
33. Vongnam T, Wittayalertpanya S, Raungrungsi N, Limpanasithikul W (2013) Inhibitory effect of *Derris reticulata* ethanol extract on LPS-induced macrophage activation. Asian Biomed 7:89–95. doi:10.5372/1905-7415.0701.154
34. WHO (1980) Expert committee on diabetes mellitus. Technical Report Series 646. World Health Organization, Geneva, p 80
35. Zhang L, Yang J, Chen XQ, Zan K, Wen XD, Chen H et al (2010) Antidiabetic and antioxidant effects of extracts from *Potentilla discolor* Bunge on diabetic rats induced by high fat diet and streptozotocin. J Ethnopharmacol 132:518–524