



***Graptophyllum pictum* (L) Griff Leaf Extracts Have Potential to Protect Pancreas of Alloxan-induced Hyperglycemic Mice**

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Abstract : Background: Antidiabetic compounds in plants provide important sources for the development of new drugs in the treatment of diabetes mellitus. *Graptophyllum pictum* (L) Griff is a plant believed to have potential for alleviating symptoms of diabetes mellitus. The purpose of the present study was to evaluate the activity of *Graptophyllum pictum* (L) Griff leaf extracts in protecting pancreatic cells of alloxan-induced hyperglycemic mice.

Methods: Extracts of *Graptophyllum pictum* (L) Griff leaf were obtained by macerating the plant leaf with ethanol and then partitioning the extract with diethyl ether, ethyl acetate, and butanol. Resultant extracts were used to treat hyperglycemic mice over 14 days.

Results: The results showed that the leaf extracts have the ability to protect the pancreas from alloxan-induced damage. An ethyl acetate extract showed the highest protective activity.

Conclusion: The *Graptophyllum pictum* (L) Griff leaf extract has the potential to be developed as a source for anti-diabetic medication.

Keywords : Antidiabetic agent, *diabetes mellitus*, *Graptophyllum pictum* (L) Griff, hyperglycemic mice.

Introduction

Diabetes mellitus is a chronic metabolic disorder resulting from defective insulin production, defective insulin action, or both. The signs and symptoms of diabetes are often neglected because the consequences of hyperglycaemia are not manifest immediately [1]. Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. This reflects an increase in associated risk factors such as being overweight or obese. Over the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries [2]. There are two main types; type 1 and type 2 diabetes mellitus. In type 1 diabetes, there is an absolute loss of the insulin-producing cells (β cells) in the pancreas, and insulin is required for survival [3]. Type 2 diabetes is a complex metabolic disorder associated with developing insulin resistance, impaired insulin signaling and β -cell dysfunction, abnormal glucose and lipid metabolism, sub-clinical inflammation and increased oxidative stress [4]. Type 2 diabetes mellitus is generally managed through a stepwise program of intensive therapy which consists of lifestyle and sequential addition of oral antihyperglycemic agents and insulin as necessary [5].

Plants are a traditional sources of medicine. It is estimated that about 800 plants may possess antidiabetic potential [6, 7]. Plant-derived curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), for example, has been shown to have pancreatic protective effects in alloxan-induced diabetic rats. It

improved the histopathology and ultrastructure changes of pancreatic islets, alpha cells and exocrine acini, which reverted to a state close to their normal structure. Moreover, it increased insulin immunoreactivity and decreased elevated glucose concentrations [8]. We have previously shown that an extract of *Graptophyllum pictum* has inhibitory activity on α -glucosidase activity [9] and is able to reduce the blood glucose level of alloxan-induced hyperglycemic mice [10]. Therefore this study was conducted to evaluate the activity of *G. pictum* extract in protecting pancreatic cells of alloxan-induced hyperglycemic mice.

Materials and Methods

Preparation of plant extracts

The *G. pictum* extract used in the present study was from the study of Rahmi et al. [10]. We have previously reported the procedure for extract preparation [9, 10]. Briefly, fresh leaves of *G. pictum* collected from Biopharmaca Research Center, Bogor Agricultural University, were washed with water, cut into small pieces and sun-dried for 5 days (moisture: < 10%). They were then ground into powder form (size: 80 mesh). Extraction was carried out by maceration with 96% ethanol (ratio of leaf powder: solvent was 1:10) for 24 hours. The mixture was filtered and the filtrate obtained was collected and evaporated with a rotary evaporator at 40°C. The ethanol extract was dissolved in water, shaken and extracted by partitioning with a separating funnel, first with diethyl ether, followed by ethyl acetate and finally with butanol. Each filtrate was evaporated on a rotary evaporator to generate diethylether, ethylacetate, and butanol extracts, respectively.

Treatment of experimental animals and histopathological examination.

The experimental animals were male mice around 2-3 month-old weighing 140-210 g. Hyperglycemic mice were generated by alloxan-intraperitoneal-injection (200 mg/kg body weight). Thirty five mice were divided into 7 groups (A, B, C, D, E, F, G), five mice for each group, with the treatment for each group as in Table 1:

Table 1. Treatment of experimental animals

Group	Treatment
A (normal)	Non-alloxan treated group, given 1 mL of 5% Tween 80
B (positive control)	glibenclamide 0.25 mg/kg body weight in 1 mL 5% Tween 80
C (negative control)	1 mL of 5% Tween 80
D	ethanol extract, 50 mg/kg body weight in 1 mL of 5% Tween 80
E	diethyl ether extract, 50 mg/kg body weight in 1 mL of 5% Tween 80
F	ethyl acetate extract, 50 mg/kg body weight in 1 mL of 5% Tween 80
G	butanol extract, 50 mg/kg body weight in 1 mL of 5% Tween 80

Experiments were carried out over 28 days, consisting of 14 days of adaptation and 14 days of treatment. Histological examination of the pancreas was carried out with light microscopy. Specimens were fixed with 10% neutral buffered formalin (BNF), embedded using paraffin and then cut using a rotary microtome. The preparations were stained with haematoxylin-eosin (HE) and Mayer's haematoxylin. After washing the each preparation was dried and then examined under the light microscope.

Results

The histopathological appearance of the pancreatic tissue of each group of experimental mice are depicted in Fig 1.

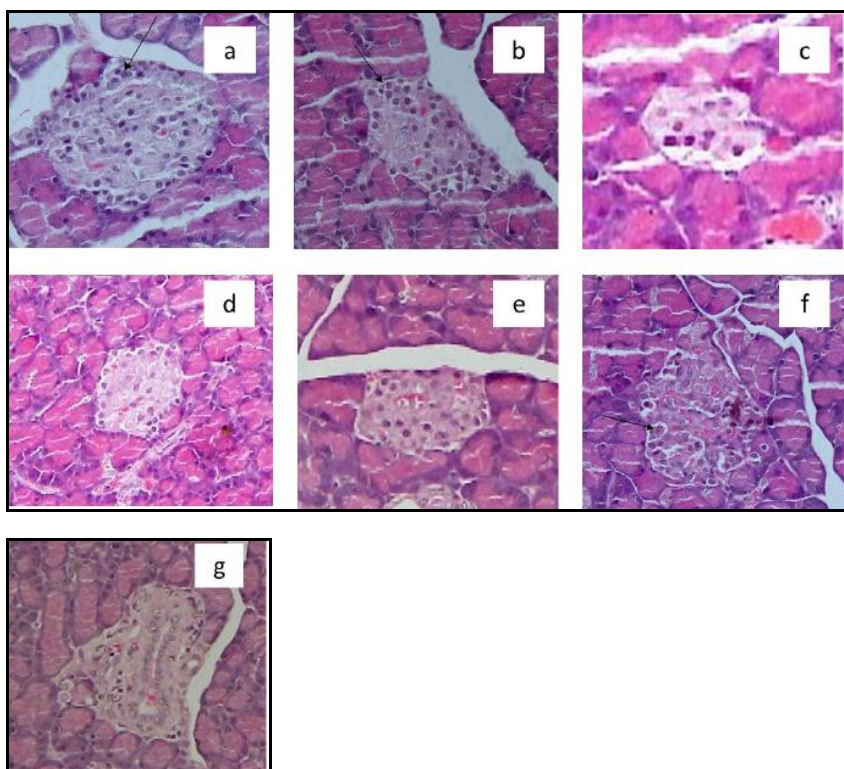


Figure 1. Histopathology of pancreatic tissues of the experimental mice. a = normal group (non-alloxan treatment, 1 mL of 5% Tween 80) ; b = positive control (glibenclamide 0.25 mg/kg BW); c = negative control (1 mL of 5% Tween 80); d = ethanol extract (50 mg/kg BW); e = diethyl ether extract (50 mg/kg BW); f = ethyl acetate extract (50 mg/kg BW); g = butanol extract (50 mg/kg BW).

As shown in Fig 1a the normal group the Langerhans islets are large and numerous with normal appearance and no lesions. In Fig1b in the positive control group the Langerhans islets are also large but the number are slightly reduced; In Fig 1c the negative control group the number of Langerhans islets are lowest; In Fig 1d the group treated with ethanol extract, the Langerhans islets are more than those found in negative control but still less than those found in the normal group; In Fig 1e the group treated with diethyl ether extract, the Langerhans islets are greater than those found in negative control but still less than those found in normal group; In Fig 1f the group treated with ethyl acetate extract, the Langerhans islets are more than those found in negative control but still less than those found in the normal group. Among the extract-treated groups, this group has the highest number of Langerhans islets; In Fig 1g the group treated with butanol extract, the Langerhans islets are higher than those found in negative control, but still less than those found in the normal group.

Discussion

We report the pancreatic-protective-potential of leaf extracts of *G. pictum* (L) Griff on alloxan-induced hyperglycemic mice. As shown in Fig 1, groups of the hyperglycemic mice treated with *G. pictum* (L) Griff extract showed improvement of pancreatic tissue structure and the number of Langerhans islets. Among the extract treated groups, those treated with ethyl acetate extract showed the best pancreatic tissue recovery indicated by the highest number of Langerhans islets with normal appearance. This indicated that the ethyl acetate extract of *G. pictum* (L) Griff leaf has the best protective effect against alloxan-induced pancreatic damage. The pancreatic histopathology of mice treated with ethyl acetate extract are nearly reverted to normal. This suggests that the bioactive compounds present in this extract may inhibit the alloxan-induced necrosis of the β cells and inhibit the free radicals production. Alloxan has been indicated to cause necrosis of pancreatic β -cells and induce free radicals generation [8].

We have also tested the blood glucose lowering activity of all types of extract used in this study. Consistent with the current finding, the ethyl acetate extract of *G. pictum* (L) Griff showed the highest activity,

bringing about a decrease of blood glucose level of 37.6% [10]. Ethyl acetate is a moderately polar solvent commonly used as extraction solvent in pharmaceutical industries. The ethyl acetate fraction of *Chaenomeles sinensis* was also found to have a very good antidiabetic effect [7].

Glibenclamide, a second-generation sulfonylurea, was used as a positive control in this study. As expected, glibenclamide treatment resulted in improvement of pancreatic tissue indicated by the increase in the size of the Langerhans islets. Previous study has reported that glibenclamide could increase insulin production by the β cells. Increasing doses of glibenclamide, however, does not produce a proportional increase in insulin secretion or a proportional decrease in blood glucose concentration. Increasing doses beyond a critical level, in a given individual, may even paradoxically worsen the glycemic control [11].

In Indonesia, the leaves of *G. pictum* (L) Griff, are traditionally used as a medicine for the treatment of constipation, rheumatism, menstruation, hemorrhoids, urinary infections, scabies, swelling, maturing boils, smoothing skin, wounds, dermatitis, hepatomegaly, ear disease, laxative, and chancre. Phytochemical analysis revealed that *G. pictum* (L) Griff contains tannins, steroids, and alkaloids [9]. The essential oils of this plant have also been shown to have cytotoxic, antioxidant and antibacterial activities. The major components found in the oils were phytol, *n*-nonacosane and hexahydrofarnesyl acetone [12].

The present study developed a strategy to protect the pancreatic β cells using various extracts of *G. pictum* (L) Griff. The pancreatic β cells were targeted because they been considered as a key player in the pathogenesis of both type 1 and type 2 diabetes. Improved glucose homeostasis can be achieved by preserving, expanding and improving the function the pancreatic β cells [13].

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