Antidiabetic Potential of Methanol Extract of Flamboyant (Delonix regia) Flowers

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Abstract. Providing scientific evidences for the medicinal benefits and possible toxic effects of the flamboyant (Delonix regia (Bojer ex Hook.) Raf.) flowers is very important to implementing the medicinal plant in this modern era. This study aimed to investigate antidiabetic potential of methanol extract of flamboyant flowers using a completely random design. Twenty-four male rats were randomly divided into 6 treatment groups with 4 replications: healthy rats (normal control, P1), diabetic rats treated with flamboyant flower extract of 0 (negative control, P2), 100 (P3), 200 (P4), and 400 mg/kg BW (P5), and rats which were induced by 0.45 mg/kg BW of Glibenclamide (positive control, P6). Diabetic condition was achieved by a single injection of alloxan 150 mg/kg BW. Treatments were given once a day for 14 days. On day 0, 3 and 18 blood samples were withdrawn from rats' orbital vein for glucose measurement.

All rats were sacrificed for liver, gastrocnemius muscles and pancreatic tissues collection. The liver and gastrocnemius muscle were subjected for glycogen measurement whereas pancreatic tissues were processed for histological examinations. Data was analyzed by ANOVA and followed by Duncan test. The results showed that flamboyant flowers extracts significantly (p<0.05) reduced blood glucose as well as degeneration and necrosis of pancreatic β cells. Optimal dose to decrease blood glucose and pancreatic cell degeneration was 200 mg/kg BW; whereas optimum dose to decrease pancreatic cell necrosis was 400 mg/kg BW. In conclusion, flamboyant flower extract can reduce blood glucose in rats. This is the first that shows antidiabetic potential of local flamboyant flower extracts along its toxicity effect to pancreatic tissues. These informations could become a basic consideration for the use of the plant extracts as a candidate to cure patients with diabetic problems.

Key words: blood glucose; Delonix regia flowers; diabetes mellitus; glycogen; histopathology


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INTRODUCTION

Diabetes mellitus (DM) is a chronic hyperglycemic state due to hormonal disorders. The cause of DM is the decrease of insulin hormone produced by β cells of the pancreas that play a role in cell glucose metabolism, causing higher levels of blood glucose and hyperglycemia (Suarsana et al. 2010). Hyperglycemia leads to an increase of free radicals in cells, which in excessive amounts can be toxic and encourage oxidative stress, a metabolic condition that can exacerbate the damage of pancreatic β cells (Chaiyasut et al. 2011).

Based on the World Health Organization (WHO 2016) in 2012 DM was the cause of death of 1.5 million people globally. The International Diabetes Federation (IDF) stated that the global prevalence of DM in 2014 amounted to 8.3% of the total population in the world and increased in 2015 to 387 million cases. Indonesia, which has a total diabetic patient around of 8.5 millions, ranks 7th after China, India, United States, Brazil, Russia, and Mexico (IDF 2015).

The common treatment for DM is through pharmacological therapy, by using oral medications and insulin injections. The use of oral medications may cause side-effects such as headache, dizziness, nausea, bloating, flatulence, diarrhea, anorexia, hypoglycemia, liver toxicity, increased body weight, physconia (stomach enlargement), vitamin B12 deficiency, lactic acidosis, and increased risk of cardiovascular diseases (Alethea & Ramadhian 2015). Notice the risks of these side effects, this research focused on a medicinal plant as a safer alternative in the treatment of various diseases including diabetes (Oguntibeju 2019).

One of the medicinal herbals known to have antidiabetic potential is flamboyant flower (Delonix regia) (Shiramane et al. 2011). The plant contains high concentration of flavonoids, phenolic (Shanmuka et al. 2011), carotenoids and anthocyanins (Veigas et al. 2012). Flavonoids and phenolic compounds can be used as antidiabetic agents (Nurhidajah et al. 2017). Carotenoids and anthocyanins in flamboyant flowers are potent antioxidants (Nurhidajah et al. 2017). The four compounds have the potential to improve heart function (Wang et al. 2016), lower blood glucose levels (Lu et al. 2021; Al-Ishaq et al. 2019), and to
cure damage of pancreatic β cells of people suffered from diabetes mellitus (Yeon et al. 2015).

The use of flamboyant flowers as an antidiabetic agent has not been comprehensively studied. Previous research by Rahman et al. (2011) has showed the ability of methanol extract of the flamboyant leaves to reduce increased blood glucose level in hyperglycemic mice. Another study by Chaturvedi et al. (2014) has confirmed antidiabetic potential of aqueous suspension of flamboyant flower leaves extract (100 and 200 mg/kg) in alloxan-induced hyperglycemic rats. The toxicity potential of phytochemicals isolated from petroleum ether and dichloromethane fractions of methanolic extract of flamboyant stem bark was already identified by Jahan et al. (2010). Based on above description, it is important to investigate the antidiabetic potential and cytotoxicity of flamboyant flowers found in Indonesia. Focussing on the blood glucose, tissue glycogen content and pancreatic tissue histology, the present study was carried out to investigate the effect of extract of flamboyant flowers found in Banda Aceh on diabetes using alloxan-induced diabetic rat model. Results obtained may provide a helpful information in the way to study the antidiabetic exploration of the plant extract for the development of antidiabetic formulation.

METHODS

Research design and treatment

This laboratory experimental study used a completely randomized design (CRD) consisting 6 treatments and 4 replications. Twenty four male Wistar rats aged 3 months with average body weight ($\bar{X} \pm SD$) of 197.00 $\pm$ 4.12 g were acclimated for 7 days before randomly assigned into the treatment groups. The rats were then fasted for 12 hours and weighed. Whole blood was collected for glucose measurement using EasyTouch GCU. All rats, except for the untreated negative control (P1), were given an intraperitoneal injection with 150 mg/kg BW of alloxan (diluted in 1 ml of 0.9% NaCl) to induce diabetic condition. Fasting blood glucose was measured on day 0 (pretreatment) and day 3 to confirm diabetic condition. Rats were then given 1 mL of distilled water (P2), methanol extract of flamboyant flower 100 mg/kg BW (P3), 200 mg/kg BW (P4) and 400 mg/kg BW (P5) or Glibenclamide 0.45 mg/kg BW (positive control, P6) per oral once a day for 14 consecutive days. On day 18, blood glucose levels were measured. All rats were sacrificed for liver, gastrocnemius muscle and pancreas collections.

Flamboyant flower extraction

Methanol extract of flamboyant flower was prepared by maceration according to protocol of Pasaribu et al. (2015). In brief, fresh flamboyant flowers were collected from Banda Aceh. Petals were separated, air-dried, and mashed into simplicia. Next, the simplicia powders (500 g) were soaked in 1.5 liters of absolute methanol for 3 x 24 hours and filtered using a filter paper. The filtrate was stored in a dark bottle container and concentrated using a rotary evaporator at 55 °C at a speed of 80-90 rpm. The extracts were then weighed and stored in a sealed container.

Measurement of glycogen content

Fresh liver and gastrocnemius muscle were collected from each mouse and weighed. All samples were dried at 60 °C for 10 days and separately ground into powder. Thirty-five mg of powders were added into 1 mL of KOH 30% and vortexed. The mixture was incubated at 95 °C for 20 minutes, cooled using taping water, and added with 1.5 mL of cool ethanol 95%. After a 20 minute centrifugation at 2500 rpm, the precipitate was diluted in 1 mL distilled water, added with 3 mL of 0.2% anthrone-sulfuric acid and vortexed. Absorbance was read using a spectrophotometer at λ 620 nm. Glycogen content was determined based on a standard curve created using serial glycogen standards (Modified from Suarsana et al. 2010).

Histological preparation

A standard histological protocol using Gomori’s chromium hematoxylin staining system was used to prepare pancreas histological slides (Baskin, 2015). The processes began with material fixation in Bouin’s solution, serial dehydration in ascending concentrations of ethanol, and clearing in xylol, continued with infiltration and embedding in paraffin, and cut at 5 µm. Preparations were then immersed in potassium permanganate and bisulfate solutions before stained with Gomori’s chromium haematoxylin. Slides were finally observed using a Olympus light microscope with 10x40 magnifications. Parameters observed were degeneration and necrosis of pancreatic β-cells.

Data analysis

Data were analyzed by One-Way ANOVA using SSPS software version 21 for Windows followed with Duncan’s Multiple Range Test (DMRT) at significance of 0.05 levels.

Research ethics

All experimental protocols used in this study have been approved by the Ethical Committee for
Blood glucose levels of rats in P1 (healthy rats, normal control) were significantly different (P<0.05) from those in 5 other treatment groups (alloxan-induced diabetic rats). However, there was no difference in blood glucose levels was observed among alloxan treated rats (P2, P3, P4, P5, and P6). Average blood glucose levels of normal control rats (P1) were also significantly different (P<0.05) from those of alloxan treated rats receiving no treatment (P2), flamboyant flower extract of 100 mg/kg BW (P3) or Glibenclamide 0.45 mg/kg BW (P6). No significant difference was identified between P1, P4 and P5 group rats. The administration of different doses of flamboyant flower extracts resulted in decreased blood glucose levels comparable to those caused by the administration of Glibenclamide.

Alloxan administration at a dose 150 mg/kg BW (P2) resulted in increased blood glucose levels, decreased proportion of degeneration and an increase in necrosis of β-cells of pancreas compared to normal control rats (P1). This condition is due to the mechanism of action of alloxan that damages pancreatic β cells. King (2012) stated that alloxan enters β cells in the same way as glucose. Alloxan has a structure similar to glucose making glucose transporter 2 (GLUT2) in pancreatic β cells recognize it as glucose. Hence, alloxan produces free radicals and cause β cells to break down, causing increased blood glucose levels (King, 2012).

Rats in the treatment groups of P3, P4, and P5 had a decrease in blood glucose and pancreatic β-cells degeneration and necrosis levels compared to those in P2. This is due to the presence of flamboyant flower extracts containing high antioxidant compounds. Flamboyant flowers contain antioxidant compounds such as tannins, saponins, flavonoids, steroids, phenolics, alkaloids, and carotenoids that have the potential to repair cell damage due to oxidative stress and their ability to control blood glucose levels (Rizky 2015; Adje et al. 2012; Shanmuka et al. 2011). Flavonoids, in addition to repairing cellular damage (Eleazu et al. 2014) may also stimulate insulin secretion, increase insulin sensitivity to glucose (Dheer & Bhatnagar 2010), modulate the activity and expression of enzymes involved in carbohydrate metabolism (Brahmachari 2011; Bahadoran et al. 2013), inhibit the absorption of excess glucose and fructose from the gut (Rizky 2015) and stimulate peripheral glucose utilization by increasing glycolytic and glycogenic pathways and simultaneously suppressing glycogenolysis and gluconeogenesis pathways (Winarsi et al. 2013).

Treatment of P6 caused a decrease in blood glucose, but still higher than the normal range of control. Glibenclamide effectively lowers blood glucose levels by stimulating insulin release from β cells and improving insulin response to glucose (Noipha & Ninla-Aesong 2018). Glibenclamide administration provides an effect on cell damage repair, but the effect cannot become normal conditions. Glibenclamide administration (antidiabetic drug) reduces cell damage due to alloxan induction and leads to degeneration of cell changes and a decrease in necrotizing cells (Rahman et al. 2011). However, the potential for cell repair by Glibenclamide in P6 treatment has not been able to match the rate of cell damage (necrosis) caused by alloxan administered.

Liver glycogen in P2 is significantly different from those in P1. This is caused by the acute diabetic

### RESULTS AND DISCUSSION

#### Blood Glucose and Tissue Glycogen

The results of blood glucose levels measurement on day 0, 3 and 18 are presented in Table-1 whereas liver and gastrocnemius muscle glycogen contents are showed in Table-2.

**Table 1.** Fasting blood glucose (x± SD) of rats at various treatment groups

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-0</td>
</tr>
<tr>
<td>P1</td>
<td>60.25± 5.50</td>
</tr>
<tr>
<td>P2</td>
<td>67.00± 7.83</td>
</tr>
<tr>
<td>P3</td>
<td>62.75± 6.50</td>
</tr>
<tr>
<td>P4</td>
<td>69.25± 6.90</td>
</tr>
<tr>
<td>P5</td>
<td>64.00± 3.92</td>
</tr>
<tr>
<td>P6</td>
<td>65.25± 8.18</td>
</tr>
</tbody>
</table>

Numbers followed by different letters in the same column state significant difference at α of 0.05 (Duncan). P1 = normal control, P2 = negative control, P3 = rats given flamboyant flower extract 100 mg/kg BW, P4 = rats given flamboyant flower extract 200 mg/kg BW, P5 = rats given flamboyant flower extract 400 mg/kg BW, P6 = positive control.
condition suffered by P2 group as indicated by increased blood glucose level up to 452.00 ± 8.80 mg/dL on day 18 (Table-1). Glycogen content in the liver and \textit{gastrocnemius} muscle of P6 rats was higher than those of P1. This probably occurred due to the effect of Glibenclamide active compounds that are able to decrease blood glucose content and to inhibit glycogenolysis (Erejuwa et al. 2011).

**Table 2.** Average level ($\bar{X} \pm SD$) of glycogen in the liver and \textit{gastrocnemius} muscle of rats in treatment groups

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Liver (µg/mL)</th>
<th>Gastrocnemius muscle (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>130.09$^{d}$ ± 4.63</td>
<td>69.20$^{b}$ ± 1.42</td>
</tr>
<tr>
<td>P2</td>
<td>68.21$^{c}$ ± 3.54</td>
<td>30.27$^{a}$ ± 4.38</td>
</tr>
<tr>
<td>P3</td>
<td>90.80$^{b}$ ± 1.94</td>
<td>55.67$^{a}$ ± 1.36</td>
</tr>
<tr>
<td>P4</td>
<td>93.53$^{c}$ ± 4.41</td>
<td>58.53$^{a}$ ± 7.67</td>
</tr>
<tr>
<td>P5</td>
<td>92.86$^{b}$ ± 3.82</td>
<td>57.63$^{a}$ ± 6.12</td>
</tr>
<tr>
<td>P6</td>
<td>143.26$^{d}$ ± 4.34</td>
<td>73.13$^{a}$ ± 4.27</td>
</tr>
</tbody>
</table>

Numbers followed by different letters in the same column state significant difference at $\alpha$ of 0.05 (Duncan). P1 = normal control, P2 = negative control, P3 = rats given flamboyant flower extract 100 mg/kg BW, P4 = rats given flamboyant flower extract 200 mg/kg BW, P5 = rats given flamboyant flower extract 400 mg/kg BW, P6 = positive control.

Administration of flamboyant flower extracts (P3, P4, and P5) resulted in decreased levels of liver and \textit{gastrocnemius} muscle glycogen compared to that of P1 and P6, but higher glycogen levels than that of P2. Decreased glycogen levels that occurred by the administration of flamboyant flower extracts might be caused by active compounds contained in the extract are unable to prevents the use of glycogen (glycogenolysis) in both liver and muscle. Glibenclamide administration (P6) increases glycogen level in the liver and \textit{gastrocnemius} muscle higher than those caused by the administration of 100, 200 and 400 mg/kg BW of flamboyant flower extracts (P3, P4, and P5). Although there was no difference in the liver and \textit{gastrocnemius} muscle glycogen observed among P3, P4, and P5, the administration of flamboyant flower extracts ranged from 100-400 mg/kg BW for 14 days might result in significantly lower liver glycogen than those in P1.

The aforementioned conditions might occur due to different mechanisms of action between Glibenclamide and the extract in reducing blood glucose and in maintaining glycogen levels in the liver and \textit{gastrocnemius} muscle of rats. Glibenclamide is an oral hypoglycemic medicine that effective in controlling glycemic conditions in the early stage of diabetes, but ineffective in preventing organ damages mediated by reactive oxygen species (ROS) (Pandarekandy et al. 2017). This is contrary to, quercetin and flavonoid compounds contained in flamboyant flower extract that have protective effects against progressive damages caused by alloxan (Dheer & Bhatnagar 2010). Quercetin also has an inhibitory effect on glycogen degradation in the liver. The inhibitory effect might directly reduce the release of glucose in the liver and might result in lower blood glucose (Peng et al. 2017).

**Degeneration and necrosis of pancreatic β-cell**

As presented in Figure-1, the administration of treatments might result in degeneration and necrosis of pancreatic β-cells. Administration of alloxan 150 mg/kg BW without any treatment (P2) caused a significant decrease (P<0.05) in the proportion of degeneration but showed a significant increase (P<0.05) in the proportion of β cell necrosis compared to treatment P1, P3, P4, P5, and P6. Treatment P4 and P5 showed a decrease in degenerative and necrotic β cells compared to P3 and P6. Treatment P3, P4, P5, and P6 showed a decrease in severe cell necroses compared to P2 still unable to match normal conditions in cellular necrotic level (P1).

![Figure-1](image)

**Figure-1.** Average proportion of degeneration and necrosis of pancreatic β cells at various treatments. P1 = normal control, P2 = negative control, P3 = flamboyant flower extract dose 100 mg/kg BW, P4 = flamboyant flower extract dose 200 mg/kg BW, P5 = flamboyant flower extract dose 400 mg/kg BW, P6 = Positive control.

The antioxidant compounds contained in flamboyant flower extracts are thought to repair cell damages from necrosis, but not its degeneration. According to Engler (2004), flavonoids (types of isoflavones, especially genistein) can increase the expression of cyclin D1 that plays an important role in regulating the growth of β cells, thus repairing damaged cells. Flavonoids can also stabilize the membrane by reducing membrane fluidity. Harborne
and William (2000) stated that certain types of flavonoids, especially quercetin, can repair damaged β cells. Flamboyant flower extract contains quercetins (Ittagi et al. 2011) that are able to stabilize blood glucose and repair pancreatic β cells by increasing antioxidant enzymatic processes in the body. Quercetin is one of the strongest antioxidant flavonoids and is often used as an antidiabetic agent (Fang et al. 2008). Quercetin can inhibit the activity of α-glucosidase, a key enzyme contributes to breaking carbohydrates in the diet into glucose (Hussain et al. 2012). Quercetin suppresses postprandial hyperglycemia by inhibiting active transport of glucose by sodium-dependent glucose transporter (SGLT1) and its facilitated transport via glucose transporter (GLUT2) in the intestine (Cermak et al. 2004). Quercetin improves insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes (Fang et al. 2008), improves insulin sensitivity by increasing tyrosine phosphorylation of insulin receptors and prolonging signaling processes, all of which stimulate insulin resistance in the peripheral tissues (Kannappan & Anuradha 2009).

According to Hendrawati (2017), quercetin has the ability to reduce free radicals by increasing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase (Youl et al. 2010), thus having a protective effect on pancreatic β-cells. It has been investigated that quercetin can activate the extracellular signal-related kinase pathway (ERK 1/2) which will increase insulin secretion and protect pancreatic β cells from oxidative damage (Youl et al. 2010). Therefore, flavonoid compounds in the methanol extracts of flamboyant flowers may help repair damaged pancreatic β cells by counteracting various free radicals effect and by increasing the capability of endogenous antioxidants in the body.

Flamboyant flower extracts contain carotenoids that can help prevent cells from free radicals and repair their oxidative stress damages. These compounds play a major role in supporting human health and survival. According to Fiedor and Burda (2014), carotenoids play an important role as antioxidants, immunity enhancers, and cell regeneration. Carotenoids are able to protect cells and organisms from oxidative damage caused by free radicals. The antioxidant properties of carotenoids achieved by capturing singlet oxygen and then direct interaction of the compounds with free radicals. The inhibition effect of free radicals by carotenoids is mainly done by β-carotene compounds (Mordi et al. 2020; Akrom et al. 2014).

\[\text{Figure-2. Pancreatic β cell histology in various treatments. (A) P1= normal control, (B) P2= negative control, (C) P6= positive control, (D) P5= Flamboyant flower extract dose of 400 mg/kg BW. A = α cells, B = β cells, D = degeneration, N = necrosis. 10x40 magnifications.}\]

Carotenoid compounds of flamboyant flower extract can reduce cell damage caused by oxidative stress. According to Akrom et al. (2014), β-carotene compounds are antioxidative agents that can protect
pancreatic damage caused by alloxan. Hue et al. (2012) reported that β-carotene compounds can inhibit the formation of chelates through antioxidant mechanisms so that the cells of the Langerhans are protected from genotoxic stress. According to Winarsi et al. (2013), the β-carotene compound provides protection at the cellular level where DNA is protected against a variety of disorders so as to be protected from other compounds that destroy the genetic code and prevent mutations. Carotenoid compounds contained in the flamboyant flower extract can counteract free radicals and prevent cellular oxidation that can trigger degenerative diseases.

Besides take a role in chelating free radicals, the administration of flamboyant flower extract also allegedly can initiate releasing of stem cells in the body to get to damaged organs, and has the potential to regenerate new cells in the organ. The differentiated cells are expected to regenerate new cells in the Langerhans island of the pancreas. According to Erwin et al. (2013), an increase in the number of Langerhans β cells can occur due to the body's ability to regenerate damaged β cells. The regeneration begins with repair and cleavage of new β cells (mitosis). The decreased proportion of β-cell necrosis occurs gradually. According to Zhou & Melton (2018), 90% of pancreaticectomy in adult rats can undergo regeneration. In the remaining pancreatic tissues, there is an increase in β cell proliferation and the formation of new pancreatic lobes within 8 weeks. Guz et al. (2001) stated that in the regenerated Langerhans island there are two presumptive precursor cell types derived from the pancreatic duct epithelium, the one expresses the glucose transporter-2 (Glut-2) and the other one expresses insulin and somatostatin. The results of Brennand et al. (2007) study showed that pancreatic ductal cells have the ability to form new Langerhans islets and β cells. Therefore, pancreatic duct epithelial cells are assumed to be the primary source of stem cells for the regeneration of pancreatic β cells.

Previous studies have shown the potential of flamboyant flower extract to induce differentiation of mesenchymal stem cells (MSC) into nerve cells (Eriani et al. 2018) and osteoblasts (Eriani et al. 2020) in vitro. The MSCs, that are almost present in all tissues in the body such as bone marrow cells, adipose tissue, muscle, heart, and lung (Wei et al. 2013; Williams & Hare 2011) have potential as immunomodulators and tissue regeneration. Therefore they are widely used as a therapy for degenerative diseases such as diabetes mellitus (Chen et al. 2017). Hess et al. (2003) reported that transplantation of bone marrow cells results in localization of the Langerhans duct and structure of the island as well as increased insulin secretion. According to Lammert et al. (2001), bone marrow cells can repair damaged pancreatic endocrine cells by differentiating into endothelial cells. These cells will stimulate the proliferation of progenitor cells and their differentiation later into pancreatic endocrine cells.

Moreover, flamboyant flower extract is thought to be able to induce regeneration of damaged pancreatic β cells, a mechanism assumed facilitated by the mesenchymal stem cells. Several conducted in vitro studies above showed that stem cells can differentiate and regenerate pancreatic β cells in people with diabetes mellitus. It is therefore suspected that giving flamboyant flower extracts can initiate the release of stem cells in vivo, either in the pancreas tissue itself or in other tissues in the body to reach the pancreatic organs, then differentiate into pancreatic endocrine cells so that can regenerate β cells. However, it is still in suspicion and further researches are needed to prove it.

This study is to be the first accessing the effects of flamboyant (D. Regia) flowers extract on blood glucose and tissue glycogen contents as well as the histopathology of pancreatic tissues. The data obtained, therefore, not only provide better explanation of the effect of flamboyant flowers extract on the circulatory glucose but also on glycogen mobilization and possible pancreatic cells recovery. The present study also used smaller doses of Glibenclamide than those implemented by other previous researchers (Rahman et al. 2011; Jahan et al. 2010). The effect of this drug on both blood glucose and pancreatic cell recovery is lower compared to those caused by flamboyant flower extracts. Results obtained in this study, together with those previously presented about the effect of D. regia extract on the proliferation and differentiation of bone originated stem cells (Eriani et al. 2018) has showed possible use of the plant extract in diabetes management and therapy, an important contribution in the way to study the antidiabetic exploration of the plant extract for the development of antidiabetic formulation.

CONCLUSION

Methanol extract of flamboyant flower (Delonix regia) can reduce blood glucose and is potential for Diabetes mellitus treatment. The optimal dose for lowering blood glucose levels, maintaining liver and muscle glycogen, and reducing proportion of β cell necrosis is 200 mg/kg BW.
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