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The Antihyperglycemic Activity of Bruguiera gymnorrhiza Roots Extract in STZ-induced Diabetic Mice

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ABSTRACT

Mangrove plants are believed to possess a wide range of bioactive compounds due to their ability to thrive in harsh conditions enduring high salinity, low air humidity and high temperature. *Bruguiera gymnorrhiza* is one of the mangrove species that is commonly found in Sabah and its root has been discovered to have antidiabetic activity. However, previous investigations focused solely on ethanolic extract of *Bruguiera gymnorrhiza* root (BGR). Thus, this study aimed to assess antihyperglycemic activity of BGR methanolic extract in various fractions (aqueous, butanol, chloroform) using streptozocin-induced diabetic mice as an experimental model. The diabetic mice were administered different fractions of BGR extract at dose of 250 mg/kg with metformin (200 mg/kg) serves as standard drug. Blood glucose levels were measured on day 0th, 7th and 14th following the oral administration of BGR fractions in a fasting state mice. After 14 days treatment period, the results showed that the aqueous fraction of BGR extract significantly reduced the blood glucose levels in diabetic mice compared to other BGR fractions. The significant antihyperglycemic effect observed in aqueous fraction of BGR extract strongly indicates the presence of major potent antidiabetic components for decreasing the elevated blood glucose levels in diabetic mice.

Keywords: *Mangrove; diabetes; Bruguiera gymnorrhiza*

INTRODUCTION

Diabetes mellitus (DM) or diabetes is a group of metabolic conditions that associated with hyperglycemia due to partial or total insulin efficiency (Egan & Dinneen, 2019). Diabetes is one of the five leading causes of death worldwide that associated with many health complications such as long-term damage and failure of various organs (Chandramohan *et al*., 2008). International Diabetes Federation (2021) reported that 537 million adults diagnosed with diabetes in the range of 20 to 79 years old with approximately 6.7 million deaths estimated. Ministry of Health Malaysia conducted latest National Health and Morbidity Survey (NHMS) (2019) reported that about one in five Malaysians adult (18.3%) has diabetes.

Currently, diabetes is managed by different types of oral hypoglycaemic drugs such as sulfonylureas, biguanides, and alpha-glucosidase inhibitors that is available in market (Bader *et al*., 2017). The use of plants for medicinal purposes are being made toward its improvement. Some plant-drugs have been used for centuries, therefore, there are growing interest in medicinal plants and their bioactive molecules and central point of research. Traditional medicine from plants has been used to treat several diseases including diabetes since there are safe and available (Sathasivampillai *et al*., 2017). Insulin therapy required the patients to regularly monitor their blood glucose level and it will develop several harmful side effects (Bowker *et al*., 2006; Monami *et al*., 2009). Oral antidiabetic drugs also possess acute side effects such as liver toxicity, nephrotoxicity, heart diseases, hypoglycemic conditions, or common side effects, such as stomach-related problem and nausea (Ivemeyer *et al*., 2012). Herbal remedies are one of the approaches that could treat diabetes with less side effects. It has been reported that no side effects associated with herbal drugs (Saravana & Pari, 2006).

Mangrove is a small tree that grows in coastal brackish or saline waters in muddy or rocky soils. It is highly tolerant to salts, halophytes and quickly adapt in harsh conditions (Kathiresan & Bingham, 2001). The diversity of mangrove flora worldwide includes around 81 tree and shrub species of 30 genera from 17 families (Sachithanandam *et al*., 2019). Asia holds the biggest number of mangrove area which approximately 6.8 million ha out of 14-15 million ha (1990-2020 data) of the world's total mangrove area(Food and Agriculture Organization, 2020; Kauffman *et al*., 2011). Mangrove in Malaysia constitutes approximately 537,686 ha in area, and more than half of the total area was covered by Sabah with 364,100 ha (Olaniyi *et al*., 2012). Sabah covers the greatest mangrove area in Malaysia, yet limited amount of study has been done especially for *Bruguiera gymnorrhiza* species. Oral glucose tolerance test (OGTT) has been studied in diabetic rats with the treatment of aqueous and butanol extracts of *Bruguiera gymnorrhiza* root resulting significantly lower blood glucose level by 30% and 48.6% respectively (Singh *et al*., 2010). In this study, *Bruguiera gymnorrhiza* mangrove plant will be conducted to determine its anti-diabetic activity and its mechanisms in lowering blood glucose level.

MATERIALS & METHODS

Animals

Male Balb/c mice weighing 20g-30g were used in present study. Mice were housed in a clean cage at room temperature, 12-h light/12-h dark cycle and relative air humidity 40-60%. Mice were maintained in regularly monitored setting in quarantine room of Animal BSL-3 in Biotechnology Research Institute, Universiti Malaysia Sabah. All experiment protocols were reviewed and approved by Animal Ethics Committee, UMS with ethical code AEC 0019 / 2022. The mice used in the present study were kept in the full accordance of Researcher's Guidelines on Code of Practice for the Care and Use of Animal for Scientific Purposes, as well as standards and recommendations adhered by ethical committee.

Plant extract preparation and fractionation

The root of *Bruguiera gymnorrhiza* was air-dried and powdered using heavy duty blender. The powdered root weighed 10g and extracted via Soxhlet extraction in 90% methanol for 3 cycles. The concentrated extract was diluted in 90% methanol and fractionated with butanol and chloroform to make aqueous, butanol, and chloroform fraction extract of *Bruguiera gymnorrhiza* root.

Brine Shrimp Lethality Assay

The eggs of brine shrimp, and sea water were collected from UMS prawn hatchery. Air pump, separating funnel, eggs of brine shrimp and sea water were needed to be prepared for hatching process. Approximately 0.5g of brine shrimp eggs were added into separating funnel filled with 500 mL of sea water. The mixture was mixed well with a spatula. Hatching process took about 24 hours and to ensure a successful hatching and a production of mature brine shrimp, the lights in the setup area must be switched on throughout the process and maintain a proper aeration by placing the air pump into separating funnel. Once the brine shrimps mature, it was then called nauplii.

The aqueous, butanol and chloroform fraction extracts were dissolved in sea water and were added into 24-well plate. Each well was filled with each fraction extracts with different concentrations ranging from 50 µg/mL to 1500 µg/mL in triplicates. The final volume of each well was adjusted into 2 mL final volume of seawater and 10 living nauplii were introduced in each well. A volume of 2mL seawater and 70% ethanol were served as positive and negative control respectively with 10 living nauplii introduced. The surviving nauplii were counted after 24 hours. LC_{50} value was calculated using probit analysis in SPSS and will find the potential toxicity in plant extract.

Induction of STZ

Diabetes was induced using single high dose STZ at 200 mg/kg body weight of Balb/c strain mice via intraperitoneal injection. The concentration of the final solution is 20 mg/mL that makes 20 mg of STZ was dissolved in 1 mL of solvent. Once STZ dissolved, it must be used as soon as possible since STZ will lose its diabetogenic function in solvent and the injection process must be done within 10 minutes after dissolution. After one week of injection, the fasting blood glucose level of the mice was measured to confirm diabetes. The fasting blood glucose level was measured using OneTouch glucose meter and the mice that reached hyperglycemia (upper 11 mmol/L) were chosen for animal study.

Antihyperglycemic activity of *Bruguiera gymnorrhiza* roots fraction extracts in STZ-induced diabetic mice

The mice were isolated into six groups (Group I, II, III, IV, V and VI) of five mice each as follows, Group I served as normal control that received normal saline which is the only group that did not received any injection of STZ. Group II also received normal saline but served as diabetic control. Group III treated with metformin that act as standard drug at a dose of 200 mg/kg. The remaining groups (Group IV, V, and VI) were treated with *Bruguiera gymnorrhiza* root aqueous, butanol and chloroform fraction respectively at 250 mg/kg dosage. The treatment continued for 14 days with free access to food and water. Blood glucose levels were measured on day 0, day 7, and day 14 using OneTouch Ultra Plus machine. The mice were fasted for six hours prior to measurement of blood glucose level by housing the mice with new cage and clean bedding and removed their food access and water. After six hours of fasting, a drop of blood was drawn from the mice through tip of the tail to measure its blood glucose levels. After continuous treatment of extracts, the mice were further tested to Oral Glucose Tolerance Test (OGTT).

Oral Glucose Tolerance Test (OGTT)

The experimental animals from the previous assay were then fasted for six hours and given administration orally in their respective treatment. Group I and Group II were administered with normal saline, while metformin was treated to Group III in 200 mg/kg dosage. Group IV, V and VI were treated with aqueous, butanol, and chloroform fraction extract respectively in 250 mg/kg body weight. After 30 minutes of treatment administration, the fasting blood glucose level of each mouse was measured using OneTouch Ultra Plus Glucose Meter machine by collecting the blood through the tail tips of mice. This blood glucose level measurement indicated as 0 minutes prior to glucose (2 g/kg body weight) administration to all groups. Blood glucose levels were measured at 30 minutes, 60 minutes, and 120 minutes after glucose administration. The blood glucose level was expressed in millimole per liter (mmol/L).

Statistical Analysis

All data are presented as mean ± SEM. Differences between groups were evaluated by one-way ANOVA followed by Tukey's multiple comparisons tests, and differences were considered significant at $p < 0.05$.

RESULTS & DISCUSSION

Brine Shrimp Lethality Assay

In brine shrimp lethality assay, LC_{50} or 50% mortality rate of brine shrimp calculated and shown on Table 1. Through the LC50 value of each fraction, both aqueous and butanol fraction, is non-toxic while chloroform fraction is toxic, or medium toxic. The toxicity category was referred to according to Meyer et al., (1982), and Clarkson et al., (2004). However, the chloroform fraction of BGR that was administered to mice was in low concentration. This is supported by the positive correlation of brine shrimp lethality assay and mice acute toxicity test by Lagarto Parra et al., (2001).

Table 1 The LC_{50} value of fraction extracts of BGR

Concentration	LC_{50}
Aqueous fraction	2927.05 µg/mL
Butanol fraction	1217.76 µg/mL
Chloroform fraction	480.69 µg/mL

Antihyperglycemic activity of *Bruguiera gymnorrhiza* roots fraction extracts in STZ-induced diabetic mice

A significant reduction on fasting blood glucose levels demonstrated by diabetic mice that were treated with metformin at 200 mg/kg and aqueous fraction extract of *Bruguiera gymnorrhiza* root at 250 mg/kg. After 14 days of treatment, present data (Figure 1) showed that there is significant reduction in elevated fasting blood glucose levels by day 7 of treatment. Meanwhile, 250 mg/kg of butanol and chloroform fraction extracts of BGR showed no significant reduction in fasting blood glucose levels after 14 days of treatment. The antihyperglycemic activity shown by aqueous fraction of BGR may be due to the abundance of bioactive compounds.

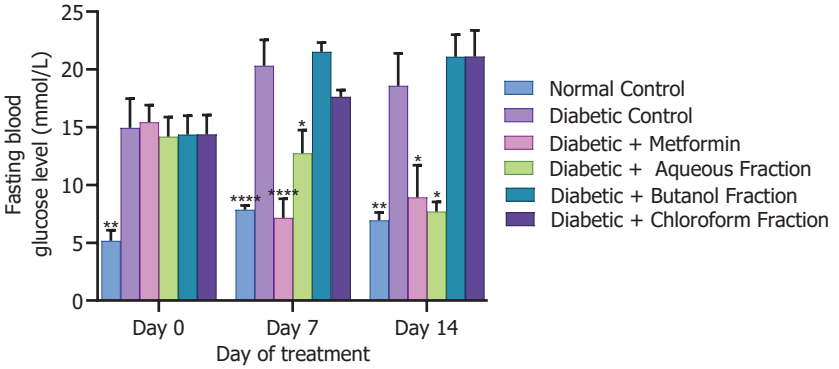

Figure 1 The antihyperglycemic effects of *Bruguiera gymnorrhiza* root fraction extracts in STZ-induced diabetic mice

Oral Glucose Tolerance Test (OGTT)

Metformin was used as a standard drug in present study, and it significantly lowered the fasting blood glucose levels of diabetic mice at 30 min, 60 min and 90 min compared to diabetic control group. Diabetic mice that were treated with aqueous fraction extract of BGR showed significant reduction at 120 min post glucose loading compared to diabetic control group. The butanol and chloroform fraction extract of BGR did not show any significant reduction in fasting blood glucose levels. This indicates the enhancement of glucose tolerance in aqueous fraction of BGR suggests that there is potent anti-diabetic properties.

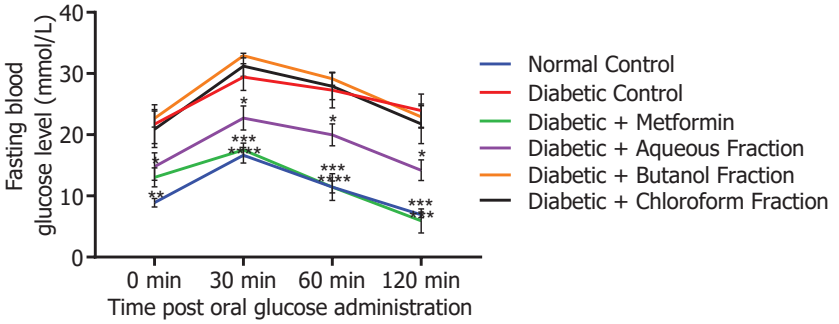

Figure 2 Effects on oral glucose tolerance test after treating *Bruguiera gymnorrhiza* root fraction extracts on STZ-induced diabetic mice

CONCLUSION

The present study has suggested that the aqueous fraction of BGR possesses anti-hyperglycemic activity. The compound isolated in aqueous fraction of BGR needs further investigations to discover its mechanism of action and to identify its active compound responsible in anti-hyperglycemic activity.

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