A PRELIMINARY STUDY: ANTIHYPERGLYCEMIC ACTIVITIES OF Phaleria macrocarpa FRUITS AND SITAGLIPTIN, AN INHIBITOR OF DIPEPTIDYL PEPTIDASE (DPP-IV)

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ABSTRACT

Phenolic compounds in plants have been experimentally proven to have activity as inhibitors of dipeptidyl peptidase IV (DPP-IV), one of enzymes responsible for the increase in blood glucose in diabetic patients. Phaleria macrocarpa contains these secondary metabolites in a large amount so that potentially show activity close to sitagliptin in improving diabetes. To evaluate this activity, we conducted a study in which ethanol extract of P. macrocarpa (EEPM) were given at dose 550 mg/kg; 1100 mg/kg; and 1650 mg/kg in alloxan induced-diabetic rats for 7 days. The positive control was given sitagliptin at dose 9 mg/kg while negative controls were fed aquadest. The result revealed that the administration of EEPM and sitagliptin attenuated blood glucose level with no significant difference (p > 0.05) which showed a promising activity of EEPM in competing sitagliptin. This result could be a start to provide an alternative DPP-IV inhibitor.

Keywords: Antihyperglycemic, dipeptidyl peptidase IV, Phaleria macrocarpa.

INTRODUCTION

Diabetes mellitus occurs due to an insufficiency in insulin production or a developed resistance which leads to the increase of blood glucose called hyperglycemia. Untreated hyperglycemia causes damage on nerves, heart, blood vascular and kidney. There are two types of diabetes comprise of type I that only can be treated with external insulin; and type II in which internal insulin is inadequate in production or cells stop to utilize insulin thus needs drugs intervention (Govindappa, 2015).

Diabetes management faces some challenges including selecting the best drug, deciding a correct combination, managing side effects and preventing insulin resistance. Additionally, effects of antidiabetic agents are variable in patients therefore a search for the best treatment should be pursued. Several antidiabetic agents targeted enzymes that are involved in the regulation of glucose for diabetes treatment, such as amylase, glucosidase, aldose reductase and DPP-IV. Alpha-amylase and glucosidase stimulate the breakdown of carbohydrates in the absorption site. Inhibition of enzymes reduces the amount of glucose in bloodstream along with increase of insulin production. Aldose reductase prevent the conversion...
of glucose to sorbitol that induce cataract in eyes (Tiwari et al., 2014). The release of insulin is affected by action of glucagon like peptide-1 (GLP-1). GLP-1 stimulates the release of insulin, prevent the release of glucagon, increases mass of pancreas and delay gastric emptying. Degradation of GLP-1 is catalyzed by DPP-IV. Inhibition of DPP-IV result in improvement of diabetes (Sharma et al., 2015).

There have been growing interests about natural origins that exhibit activity against glucose regulating enzymes. One of them is *P. macrocarpa* or mahkota dewa fruits. *P. macrocarpa* fruits showed antihyperglycemic effects by interrupting the activity of glucose regulating enzymes. Methanol extract of phaleria showed inhibiton against α-glucosidase 20% higher than acarbose, a standard drug. Activity against amylase less effective compared to its activity against glucosidase (Ali et al., 2012).

Phenolic compounds including flavonoid were found to inhibit enzymes. Determination using Fourier transform infrared spectroscopy (FTIR) showed that magniferin and phalerin are two metabolites found in *P. macrocarpa* fruits and showed inhibition against α-glucosidase and amylase using, while others are under investigation (Easmin et al., 2017). Since polyphenols was proven to show activity related to GLP-1 by affecting signal transduction for insulin secretion (Avila et al, 2017), *P. macrocarpa* fruits enriched by phenolic compounds could possibly showed activity against DPP-IV.

**MATERIALS AND METHODS**

**Preparation of Ethanolic Extract of *P. macrocarpa***

Five kilograms of red fruits were peeled off and separated from seeds. Then fruits were cut into small pieces, dried (at 27 ± 5 °C for 5 days) and grounded into powder. The powder (200 gr) was soaked with 1,500 mL ethanol 70%. After 24 hours, the mixture was filtered and filtrat was separated from solvent. A 1,500 ml fresh ethanol was used for the second soaking. The third soaking process was repeatedly on the next day. The collected filtrat was evaporated using rotary evaporator at 60°C which yield viscous extract.

**Animals**

Wistar rats *Rattus norvegicus* (150-250 gr) were purchased from Veterinary Faculty, Syiah Kuala University. The rats were housed in a room temperature (27 ± 5 °C, 12 h-light and 12 h-dark cycle, given standard pellet twice a day and water ad libitum. The protocol was approved by Ethical Committee of Medical Faculty, Syiah Kuala University.
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**Drugs and Chemicals**

Sitagliptin were purchased from a pharmacy in Banda Aceh. Chemicals were obtained from chemicals distributors in Banda Aceh.

**Induction of diabetes in rats**

Animal were fasted for 16 hours before collection of blood glucose was taken. Alloxan 120 mg/kg was injected intraperitonially and blood glucose was recorded after 7 days of injection. Rats with blood glucose level > 150 mg/dl were selected for treatment.

**Evaluation of antihiperglycemic activity of *P. macrocarpa***

Rats were divided into 5 groups (n=5) and received treatment as follows: Group 1: N, normal control received pellet and tap water; Group 2: P, diabetic rats received sitagliptin (9 mg/kg); Group 3: EEPM A, diabetic rats treated with EEPM 550 mg/kg; Group 4: diabetic rats treated with EEPM B 1100 mg/kg, and group 5: diabetic rats treated with EEPM C 1650 mg/kg. The treatment was carried out for 7 days. On day 7th, animals were fasted-overnight. Blood samples were collected from tails using lancet and examined using portable Glucose, Cholesterol, Uric acid (GCU) Nesco Multi Check (Nesco, Taiwan).

**Statistical analysis**

The values of blood glucose were presented as mean value ± standard deviation. The significance of differences was determined using analysis of varians (ANOVA) and continued with Least Significance Difference (LSD) as post hoc test using SPSS. The data were considered different significantly when p< 0.05.

**RESULTS AND DISCUSSION**

The administration of alloxan at dose 120 mg/kg induced hyperglycemia in all experimental groups (Table 1). Blood glucose remained increased in negative control during experiment. The use of sitagliptin and phaleria extract improved hyperglycemia measured on day 7 (Table 1).

The decline of blood glucose value of negative groups was significantly different to positive, EEPM A, EEPM B and EEPM C (P < 0.05). No significant difference observed among positive group and the three EEPM groups (P > 0.05). This meant the use of ethanolic extract of phaleria showed the hypoglycemic activity at same level to sitagliptin. The use of EEPM A at dose 550 mg/kg showed the highest decrease among all groups.
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(274.60 ± 90.666 mg/dl) shown in Table 1. This result confirmed the previous study, in which the activity of methanol extract of phaleria was compared to insulin, metformin and glibenclamid. The result showed that the extract potency of phaleria extract was as effective as those 3 pharmaceutical agents (Ali et al., 2012). Glibenclamide works on ATP-potassium channel of which the influx potassium cause depolarization which stimulate release of insulin, whereas metformin prevent gluconeogenesis.

Table 1. Fasting blood glucose level in treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretest</th>
<th>Posttest</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>261.40 ± 55.846</td>
<td>378.20 ± 55.997</td>
<td>-116.80±± 51.949</td>
</tr>
<tr>
<td>P</td>
<td>303.40 ± 89.523</td>
<td>115.60 ± 16.196</td>
<td>187.80±± 40.515</td>
</tr>
<tr>
<td>EEPM A</td>
<td>366.60 ± 85.967</td>
<td>92.00 ± 8.916</td>
<td>274.60±± 90.666</td>
</tr>
<tr>
<td>EEPM B</td>
<td>270.20 ± 114.001</td>
<td>70.40 ± 5.030</td>
<td>199.80±± 113.229</td>
</tr>
<tr>
<td>EEPM C</td>
<td>316.00 ± 65.311</td>
<td>78.00 ± 20.928</td>
<td>238.00±± 80.041</td>
</tr>
</tbody>
</table>

Description: the numbers with the same letters are not significantly different.

N: diabetic rats received pellet and tap water
P: diabetic rats received sitagliptin 9 mg/kg
EEPM A: diabetic rats received phaleria extract 550 mg/kg
EEPM B: diabetic rats received phaleria extract 1100 mg/kg
EEPM C: diabetic rats received phaleria extract 1650 mg/kg

There was significant decrease in glucose level observed in positive control, in which sitagliptin was used. Sitagliptin is DPP-IV inhibitors that stimulate action glucagon like peptide-1 (GLP-1) whose half-life is very short, two minutes. GLP couple to receptor protein G which allow signal transduction, involving cAMP and tyrosine kinase, thus resulted in insulin release. Administration of sitagliptin increase half-life to 5 minutes, longer than its physiology life due to the inhibition of DPP-IV (Domínguez Avila et al., 2017). Although sitagliptin is effective for diabetes, a search for alternative drugs is beneficial since responses of patients are variable.

Polyphenols has been evidenced to increase the concentration of GLP-1. Consumption of fruits which are rich in phenolic compounds increased GLP-1 and insulin thereby reduce post prandial glucose and fasting glucose (Domínguez Avila et al., 2017). In other study, phytochemical screening showed the existence of flavonoid, phenol, saponin, tannin, alkaloid and steroid/triterpenoid (Alara, Alara, & Olalere, 2016). Compounds identified in phaleria phenolic compounds including mangiferin, phalerin,
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kaempferol, myricetin, naringin, rutin and quercetin (Hendra, Ahmad, Sukari, Shukor, & Oskoueian, 2011). A study had evaluated a total content of polyphenols in *P. macrocarpa* (Scheff.) Boerl which was 186.22 to 292.86 mg/g gallic acid equivalent (Mahzir, Ain, Abd Gani, Hasanan Zaidan, & Halmi, 2018). Phenolic compounds were noted to acts as natural DPP-IV inhibitors in several studies although exact mode of action has not been investigated further. In this study, hypoglycemic effect of phaleria extract was close to sitagliptin which validated the activity of its phenolic compounds.

**CONCLUSIONS**

The use of *P. macrocarpa* extract improved hyperglycemia which was comparable to the use of sitagliptin. Phenolic compounds contained in phaleria fruits possibly play roles in antidiabetes activity and need to be investigated further.

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**REFERENCES**


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