ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF EUGENIA POLYANTHA WIGHT LEAF FROM INDONESIA IN DIABETIC RAT WISTAR STRAIN INDUCED BY ALLOXAN

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ABSTRACT

Objective: The purpose of this study is to determine the antidiabetic activity of 96% ethanolic extract of Eugenia polyantha wight (EEEP) leaves.

Methods: 25 male Wistar rats were divided into five groups. Group I was negative control (aquadest), Group II was positive control (Glibenclamide 0.45 mg/kg), and Group III, IV, and V were treated by the 96% EEEP leaves with doses of 312.5, 625, and 1250 mg/kg, respectively. Before the treatment, all rats were induced by alloxan with a dose of 150 mg/kg by intra-peritoneal. On the 3rd day after induction, rats with blood glucose level >200 mg/dL were treated by drugs or extract appropriate to the group. On the 10th day, after blood sampling, all rats were killed, and the pancreases were taken for histopathology examination. The blood glucose level on the 10th day was tested by Kruskal–Wallis test followed by the Mann–Whitney with confidence level 95%.

Results: The administration of EEEP leaves at doses of 312.5, 625, and 1250 mg/kg body weight can reduce blood glucose levels in rat induced by alloxan. The EEEP at a dose of 1250 mg/kg body weight potentially regenerates Langerhans cell induced by alloxan. This extract has potentials as an antidiabetic agent.

Conclusion: This study shows that the 96% EEEP can be developed as an antidiabetic agent.

Keywords: Eugenia polyantha wight, Diabetic mellitus, Acinans cell, Langerhans cell.

INTRODUCTION

Indonesia is the second country after Brasilia with biodiversity [1]. Leaves Eugenia polyantha wight are commonly used as a culinary supplement. Most Indonesian people use E. polyantha wight leaves to treat hypertension and cholesterol [2], diarrhea, stomach ulcers, and diabetes mellitus [3]. Leaves, roots, stems, and fruit of E. polyantha wight have antidiabetes, antihypertension, and antidiarrhea [4]. The other effects of E. polyantha wight are the extract of E. polyantha wight with the doses of 0.18, 0.36, and 0.72 g/day for 15 days can lower triglycerides on hyperlipidemic male rats Wistar strain [5].

The water-methanol extract of leaves has free radical scavenging with an effective dose of 18 mg/mL [6]. E. polyantha wight has the antioxidant effect and high total polyphenol [7]. Extracts of E. polyantha wight have antibacterial effects against Staphylococcus aureus [8], Streptococcus mutans [9], and as antifungal against Aspergillus sp., Euroticum sp., and Penicillium sp. [10]. The infusum of E. polyantha wight can increase the number of spermatozoa on Rattus norvegicus induced alloxan [11].

This research was done to examine the effect of the 96% ethanolic extract of E. polyantha wight (EEEP) leaves toward blood glucose level on rats induced by alloxan and pancreas histopathology profile.

METHODS

The materials used in this study were E. polyantha wight, alloxan (sigma) and male Wistar rats. The E. polyantha wight was obtained from Bebel village, Wonokerto, Pekalongan, Jawa Tengah, Indonesia and harvested in March 2013. The male Wistar rats were obtained from Pharmacology Laboratory, Faculty of Pharmacy, Universitas Muhammadiyah, Surakarta. The rats were 2-3 month old, weighing 150-200 g. This study was approved by the Health Research Ethics Committee of Dr. Moewardi Hospital of Surakarta, Indonesia.

Preparation material

The leaves were covered by black flannel and dried under the sun. After drying, leaves were blended into a powder. 500 g dry powder of E. polyantha wight leaves were macerated by 96% ethanol solution 3.5 L for 2 days and then were filtered. The filtrate was evaporated by vacuum evaporator to obtain a thick extract.

Antidiabetic test

25 rats were divided into five groups. Each group consists five rats. Group I was negative control (aquadest), Group II was treated by glibenclamide 0.63 mg/kg bw, and Groups III, IV, and V were treated by the 96% EEEP leaves with doses of 312.5, 625, and 1250 mg/kg bw. All rats were fasted for 8 hrs. On the 1st day, all rats were injected by alloxan at a dose of 150 mg/kg bw intraperitonially. On the 3rd day, all rats with blood glucose level >200 mg/dL were treated by drugs/extract appropriate to the group. On the 10th day, all rats blood glucose were re-measured. All blood sampling were done on lateralis vein of tails.

Histopathology examination

On the 10th day, after blood sampling all rats were killed by decapitation. The pancreases were taken for histopathology examination.

RESULTS AND DISCUSSION

The measurement of blood glucose level was done on day 0, 3, and 10. These data can be seen in Table 1.

Statistical analysis was conducted by Kruskal-Wallis test with confidence level 95%. In the Kruskal-Wallis test, on the 10th day, the p value was 0.003 (p<0.05). This means that there were differences...
in blood glucose levels of the five treatment groups. The next test was done by Mann–Whitney. The result of the Mann–Whitney test can be seen in Table 2.

After blood sampling on the 10th day, all rats were killed, and the pancreas were taken for histopathology examination. The result can be shown in Figs. 1-3. Base on Figs. 1-3, there was vacuolation in acinar cells and necrosis in islet cells Langerhans on the negative control group, but no pathological changes on glibenclamide group and EEEP group.

Base on Table 2, it can be concluded that the 96% EEEP can reduce blood glucose level on male Wistar rats. In this research, the EEEP at doses of 312.5, 625, and 1250 mg/kg bw can reduce blood glucose level on male Wistar rats strain. This research lined up with several previous researches, among others: EEEP leaves at doses of 2.62 mg/20 g bw and 5.24 mg/20 g bw can lower blood glucose levels in mice induced by alloxan [12]. Research by Putri et al., 2014 found that the infusion of E. polyantha wight can reduce blood glucose level of diabetic rats induced by alloxan, and the reduction percentage is 7-30% [13]. The mechanism of this effect does not clear. In vitro study by Lelono and Tachibana stated that the extract of methanol:water (1:1) has the effect of inhibiting alpha-glucosidase enzyme activity with inhibitory concentration 50% (IC50) 70.90 µg/mL, while the water extract with IC50 72.72 µg/mL and methanol extract IC50 91.52 mg/mL [14]. Research by Widowati, 2008 stated that E. polyantha wight leaves have the ability as an astringent that precipitated mucous membrane proteins and formed a layer that protected the intestine. This caused inhibition of glucose absorption in the intestine [15]. Leaves of E. polyantha wight were suspected to improve insulin in vitro so as to lower the blood glucose [16]. The chemical constituents in E. polyantha wight leaves among others: Phenolic, polyphenol (tannin and flavonoid) [7,17], gaxanioloid, 10-epigaxanioplid, spirafolide, santamarin [18], and volatile compound (e.g. citral) [19-20]. Flavonoids contained in the leaves are one of the classes of compounds that could be expected to lower blood glucose levels [21]. Bio flavonoid from the plant was suggested to have the antidiabetic effect [22-24]. Prunin (naringenin 7-O-β-D-glucoside) derived of flavonoid can reduce blood glucose level in diabetic rats [25]. The various flavonoids including chrysin and its derivatives, silymarin, isoquercetrin, and rutin were reported to have an antidiabetic effect [26-28].

This study suggests that the 96% EEEP potentially regenerate acinar and islet cells of Langerhans pancreas of male rat induced by alloxan.

### Table 1: Effect of 96% of EEEP on blood glucose level in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dL)</th>
<th>Day 0</th>
<th>Day 3</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Negative control (aquadest)</td>
<td>121±11.6</td>
<td>214.3±28.5</td>
<td>242.6±47.32</td>
<td></td>
</tr>
<tr>
<td>Group II: The 96% EEEP leaves dose 312.5 mg/kg bw</td>
<td>109.6±13.63</td>
<td>252.8±25.54</td>
<td>88.6±11.17</td>
<td></td>
</tr>
<tr>
<td>Group III: The 96% EEEP leaves dose 625 mg/kg bw</td>
<td>91.4±18.5</td>
<td>190.5±24.44</td>
<td>77±9.92</td>
<td></td>
</tr>
<tr>
<td>Group IV: The 96% EEEP leaves dose 1250 mg/kg bw</td>
<td>103.4±7.63</td>
<td>231.6±35.92</td>
<td>64.4±4.15</td>
<td></td>
</tr>
<tr>
<td>Group V: The 96% EEEP leaves dose 1250 mg/kg bw</td>
<td>77.8±6.05</td>
<td>185.4±27.2</td>
<td>71.2±17.71</td>
<td></td>
</tr>
</tbody>
</table>

Blood glucose expressed as mean±SD. Number of rats (n)=5. SD: Standard deviation, EEEP: Ethanolic extract of Eugenia polyantha wight

### Table 2: The results of Mann–Whitney test in blood glucose level on 10th day

<table>
<thead>
<tr>
<th>No.</th>
<th>Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aquadest versus Glibenclamide</td>
<td>0.009*</td>
</tr>
<tr>
<td>2</td>
<td>Aquadest versus Groups III</td>
<td>0.009*</td>
</tr>
<tr>
<td>3</td>
<td>Aquadest versus Groups IV</td>
<td>0.009*</td>
</tr>
<tr>
<td>4</td>
<td>Aquadest versus Groups V</td>
<td>0.009*</td>
</tr>
<tr>
<td>5</td>
<td>Glibenclamide versus Groups III</td>
<td>0.072</td>
</tr>
<tr>
<td>6</td>
<td>Glibenclamide versus Groups IV</td>
<td>0.009*</td>
</tr>
<tr>
<td>7</td>
<td>Glibenclamide versus Groups V</td>
<td>0.047*</td>
</tr>
</tbody>
</table>

*Significant difference by Kruskal–Wallis test (p<0.05). Group III: The 96% EEEP dose 312.5 mg/kg bw; Group IV: The 96% EEEP dose 625 mg/kg bw; Group V: The 96% EEEP dose 1250 mg/kg bw. EEEP: Ethanolic extract of Eugenia polyantha wight
Fig. 3: Effect of 96% ethanolic extract of Eugenia polyantha wight at a dose of 1250 mg/kg bw during 7 days on histopathology of rat pancreas. There is no vacuolation in acinar (A) and islet cells of Langerhans (B)

CONCLUSION

The 96% EEEP can be developed as an antidiabetic agent.

REFERENCES