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Activity of ethanol extract of purpleleaves *(Graptophyllumpictum*(Linn.)Griff.*)* onalloxan-induced diabetes mice

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Abstract: Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and one of the major causes of death. This study aims to determine the antidiabetic activity of ethanol extract of purple leaves (Graptophyllumpictum (Linn.) Griff.)in mice. In this study, the inductionalloxan dose of 168 mg/kgbw in male mice and divided into 5 treatment groups. Group 1 to 3 are given the extract orally each concentration 140, 210, and 280 mg/kgbw, group 4 was given glibenclamide a dose of 0.65 mg/kgbw and group 5 given Na.CMC solution of 1% w/v. The results obtained in the analysis by paired t-test and test-one-way ANOVA showed a significant decrease ($p < 0.05$). Based on the results of this study concluded that the ethanol extract of purple leaves has an activities in reducing blood glucose levels and the most effective concentration was 210 mg / kgbw with a percent decrease of 70.4%.
Keywords : antidiabetic, purple leaves(*Graptophyllumpictum)*, ethanol extract.

Introduction

Diabetes is one of the most prevalent chronic diseases in the world, it is characterized by hyperglycemia caused abnormalities in carbohydrate, fat and protein metabolism associated with relative or absolute insulin deficiency or resistance in insulin secretion or insulin action.[1,2,3]Sustained hyperglycemia leads to oxidative stress, alterations in enzyme activity protein glycosylation and several structural changes.[4]The chronic diabetes cause some disorders the function of organs such as the eyes, nerves, heart, liver, kidneys and blood vessels.[5,6]

Diabetes is a common endocrine disorder affecting over 100 million people worldwide. The World Health Organization predicts that this number may increase fivefold in the near future and the recent estimates indicate that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by 2030.[7,8]Developing countries such as Indonesia was ranked fourth in the number of patients with diabetes mellitus the world after India, China and the United States. In 2000 in Indonesia there are 8.4 million people with diabetes mellitus and estimated to be 21.3 million in 2030.[9]

Insulin and oral antidiabetic drugs is the cornerstone of therapy used in the treatment of diabetes mellitus, although these drugs are have adverse effect (ex; hypoglycemia) or diminution in response after prolonged use, cannot always maintain glycemic and prevent diabetes complications significantly.[10,11]Plants have played a significant role in maintaining and improving quality of human health for thousands of years. The use of medicinal plants is increasing because of their widespread use and for their curative effects on various diseases. Thus there is a continuing need for alternative anti-diabetic remedies with risk-benefit ratios and greater acceptability.[12,13]

Graptophyllumpictum(L.)Griff, family Acanthaceae is one of the plants that can be used as traditional medicine. In Indonesia the plant is known as purple leaves. Empirically, the people in Ambon-Maluku Indonesiause purple leaf herbs (Maluku languages:alifuru leaves) to treat various diseases, one of them is to treat diabetes mellitus.Purple leaf (*Graptophyllumpictum*(Linn.)Griff.) has also been studied by Olagbende-

Dada et al. (2011) which showed that the aqueous extract of leaves of purple can be decreased the blood glucose levels in mice indicated by a dose of 100 mg / kg BW. Where in previous studies Olagbende-Chest, et al (2009) show that saponin, tannin and flavonoid glycoside. The presence of the flavonoid extract be linked to lower blood glucose levels (Olagbende et.al, 2011). Therefore, the aim of present study was to determine the effect of ethanol extract of purple *(Graptophyllumpictum*(linn.) griff.*)* leaves on diabetes mice.[14,15]

Matherial and Methods.

Plant Material

Samples were collected from the city of Ambon Maluku the state of Indonesia. The plants were identified by Laboratory Pharmacognosy and Phytochemical, Universitas Muslim Indonesia.

Preparation of plant extract

The Sample *Graptophyllumpictum*(Linn.)Griff leaves of200 g put into container maceration, then added solvent ethanol 96% until submerged, keep it in three days in a closed container and sheltered from direct sunlight while stirring periodically. After that done filtering and remaceration as three times. The result of filter obtained then evaporated until obtained thick ethanol extract.

Phytochemical evaluation

Ethanol extract of Graptophyllumpictum (Linn) was studied for phytoconstituents such as alkaloid, flavonoid, tannin, saponin and steroid using different phytochemical tests.[16,17]

Experimental animals

The animals male balb/c strain mices (20-30 g) before starting the experiments were acclimatized for 7 days. The mices were maintained on a 12 hour light/dark. They received a diet of standardized pellets and water ad libitum.All studies were carried out using 5 mices in each group. In the uses of animals experimentation we have followed the guidelines ofthe Institutional Committee of Ethic for the Use of Animals from the University of Hasanuddin, Macazzart City the state of Indonesia.

Antidiabetic activity

Diabetes was induced by a single intraperitoneal administration of alloxan (168mg/kg) with saline solution. Before administering alloxan, we took a baseline glycemia. After twelve hours was estimated blood samples by puncture of the tail vein and fasting blood glucose levels were measured by using glucometer. Animals with a blood glucose concentration $\geq$ 200 mg/dl were considered to be diabetic.[18,19]

The tested animals were randomized into 5 groups, consisting of five animals each group. Treatments were administered orally for 21 days once a day from the increased blood glucose (induction of alloxan). Furthermore, mice were divided into 5 groups as follows: group 1, 2, and 3 are given ethanol extract of purple leaves (EEPL) doses of 140 mg/kgbw, 210 mg/kgbw and 280 mg/kgbw as the test preparation (respectively), group 4 was given a glibenclamide suspension dose of 0.65 mg/kg as a comparison, and a solution of Na CMC 1% w/v as a negative control. Measurement of blood glucose levels on day-1 (hours-3) and on day -21.

Statistical analyzes

The results are expressed as the mean ± SD for eachgroup. Statistical differences were evaluated usingpaired t-test and one way ANOVA, with a significance level of $p<0.05$.

Results and Discussion

Diabetes mellitus is a common endocrine disorder and one of the major causes of death. This study aims to determine the activity of purple leaves against a decrease in glucose levels of alloxan-induced mice. This study used a purple leaves (*Graptophyllumpictum* (Linn.) Griff), as empirically this plant has been widely used as a traditional medicine for the treatment of several diseases such as one of them is the treatment of diabetes mellitus. And based on previous research by Olagbende-Dada et al. (2011) showed that the water extract of purple leaves have an effect in lowering blood glucose levels in alloxan-induced rats.[15]

Alloxan is a commonly one substance is used to induced a diabetes animals. Alloxan can be administered parenterally, one of which was injected intraperitoneally. Based on its mechanism of action, alloxan may induce the formation of free radicals that cause DNA damage pancreatic islet, augmentation of cytosolic calcium concentration where the excessive concentration of calcium ions contribute to insulin release supraphysiological and along with the formation of free radicals cause damage to pancreatic islet.[20,21]

Glibenclamide was used as a comparison or a positive control in this study. Glibenclamide is a sulfonylurea class of oral antidiabetic that have a strong hypoglycemic effect. The major action of sulfonylureas is to increase insulin release from the pancreas. Moreover this drug bind to the SUR1 subunits and block the ATP-sensitive K^+ channel. The drugs thus resemble physiological secretagogues (*e.g.,* glucose, leucine).[22,23]

Blood glucose levels (baseline) and after administration of alloxan can be seen in Table 1, which shows for the baseline glycemia ranging from 82 to 99 mg/dL and after alloxan induced ranging from 262 to 290 mg/dL. Further, an analysis of the data using paired t-test to see the difference in blood glucose levels between baseline and after alloxan induced. Where the results of data analysis in blood glucose levels after induction of alloxan increased significantly different ($p < 0.05$) with baseline blood glucose levels. This means alloxan managed to increase blood glucose levels of mice before therapy. For the analysis of blood glucose levels after induction (alloxsan) and after therapy for 21 days (Table 2) showed that the difference was significantly different ($p < 0.05$), in which it indicates that the test preparation and the comparison can lower blood glucose levels. These results can also be seen on the graph the reduction in blood glucose levels after induction and after therapy in the treatment group (graph 1), showing all the test preparation group has reduction in blood glucose levels were significantly different with the control group Na.CMC as negative.

Table 1.Blood glucose before and after administration of diabetogenic agent (alloxan) in the study groups.

Glycemia (mg/dl	Group 1	Group 2	Group 3	Group 4	Group 5
Baseline	97.6 ± 14.4	88.6 ± 14.7	82.6 ± 9.5	91.8 ± 10.3	94.2 ± 16.8
(Day 0) Alloxan	282.6 ± 39.7*	286 ± 31.9*	289.2 ± 37.9*	282.4 ± 28.6*	267.6 ± 20.3*

n = 5
Groups 1, 2, 3, 4 and 5 are alloxan.
*** p <0, 05 compared with baseline glycemia.**

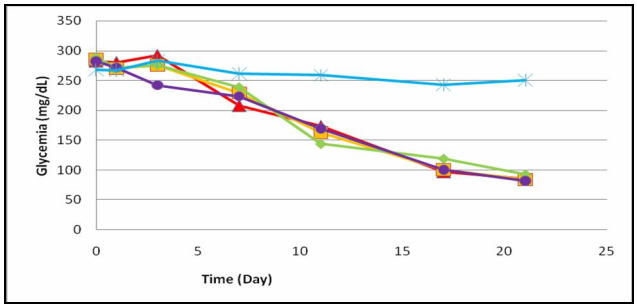
Table 2. Blood glucose in alloxan post "day 0", days 21 and % reduction

Glycemia (mg/dl	Group 1	Group 2	Group 3	Group 4	Group 5
(Day 0) Alloxan	282.6 ± 39.7	286 ± 31.9	289.2 ± 37.9	282.4 ± 28.6	267.6 ± 20.3
Days 21	85.6 ± 7.8*	84.2 ± 9.0*	93 ± 12.2*	82.2 ± 11.6*	250.2 ± 36.1*
Percentage reduction	**69.4***	**70.4***	**67.5***	**70.5***	**6.9#**

Test preparation was given after the obtained diabetic mice. The groups 1, 2 and 3 are 140, 210 and 280 mg dose/kgbw of ethanol extract of purple leaves. respectively; group 4 received glibenclamide a dose of 0.65 mg/kgbw

Intragroup

*** p <0, 05 compared with alloxanglycemia and percentage reduction**
 # p >0,05 compared with percentage reduction group 1,2,3,4



Graph 1.Graph the results of measurements of the average blood glucose levels after induction, and after therapy treatment groups. ▲ = group 1, ■ = group 2, ◊= group 3; ● = group 4, x = group 5.

The percentage reduction in blood glucose levels can be seen in Table 2, the results of the data analysis One Way ANOVA between treatment groups showed a significant ($p < 0.05$). This means there is a difference in the average percent reduction in blood glucose levels between the groups. For the test sample ethanol extract of purple leaves that has the most effective percentage reduction in a group of 2 dose of 210 mg / kg which is 70.4%, but not significantly different from groups 1, 2, and comparison (4) the percent decrease of 69.4%, 67.5% and 70.5%, respectively. This shows that the ethanol extract of purple leaves as extracts have activity in lowering blood glucose levels similar to the effect of glibenclamide suspension as comparison antidiabetics

Activity of purple leaf in lowering blood glucose levels attributable to the chemical constituents present in the extract. From the phytochemical evaluation of purple leaves (Graptophyllumpictum (Linn.) Griff) are alkaloids, flavonoids, tannins, saponins and steroids,. The reason this is in line with the phytochemical tests conducted by Olagbende-Dada et al. (2009) which states that there is chemical content of saponins, tannins, flavonoid glycosides and alkaloids in the form of non-toxic. The presence of the flavonoid extract be linked to lower blood glucose levels and hipoglycemia activities.[15,24] Flavonoids are identified to have activity as aldose reductase inhibitors.[25] (Patra*et al*.,2010). Quarcetin from flavonoids in several researches that have antidiabeticactivity and regeneration of pancreatic islets, increases insulin release in streptozocin-induced diabetes. Also reported can stimulate Ca2+ uptake from isolated islet cells.[26]

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