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Hypoglycemic Effect of Avocado Seed Extract (*Persean americana Mill*) from Analysis of Oral Glucose Tolerance Test On *Rattus norvegicus L.*

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Abstract

Due to the many side effects caused by antidiabetic drugs, it is necessary to look for alternative antidiabetic agents that have fewer side effects such as drugs from plant extracts, one of which is *Persea americana Mill* (Avocado). Ethanol extract from avocado seeds containing *polyphenols*, *tannins*, *flavonoids*, *triterpenoids*, *quinones*, *monoterpenoids*, and *sesquiterpenoids*. *Flavonoids* have shown beneficial effects against diabetes mellitus. This research was conducted to determine whether an avocado seed extract has a hypoglycemic effect. The extract concentrations used were 300, 600, 1200 mg/kg BW given orally to experimental animals (*Rattus norvegicus L.*). Rats were fed a load of 2 g/kg BW sucrose following the treatment. Blood glucose concentrations were then compared to the positive control (Glibenclamid) and the negative control (aquades). Blood glucose levels were measured from the rat's tail vein using a glucometer at -30,0,30,60,120,240 minutes after the administration of sucrose. All blood sugar levels showed decreases in concentrations following the administration of avocado seed extracts. The highest decrease in blood sugar level was observed at 300 mg/kg BW extract. Although the results of the test showed decreases in blood sugar levels, the differences in blood glucose levels before and after treatments were not found to be significant.

Keywords: Diabetes mellitus; *Persea americana Mill*; blood sugar levels; Flavonoids.

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1. Introduction

In 2017, as many as 57 million deaths occurred worldwide. Of these, 41 million deaths were caused by non-communicable diseases, mainly cardiovascular disease, cancer and chronic lung disease. Nearly three-quarters of these deaths occurred in middle- and low-income countries. The number of deaths due to non-communicable diseases is projected to increase to 52 million deaths by 2030. Diabetes accounted for 1.6 million of death due to non-communicable diseases in 2012 [1]. According to various epidemiological studies in Indonesia carried out by centers for diabetes, in the 1980s, the prevalence of diabetes mellitus in the population of aged 15 years and over was 1.5-2.3% with the prevalence in villages or rural areas lower than urban areas. The 2001's Household Health Survey found 7.5 % prevalence of diabetes mellitus in populations aged 25-64 in Java and Bali. The Basic Health Research (Riskesdas) in 2007 and 2013 found that the proportion of diabetes mellitus in 2013 almost doubled compared to 2007's [2]. Diabetes mellitus (DM) has become one of the most serious public health concerns because of the increased incidence, severe complications and the high cost of antidiabetic therapy. Considering that intensive glycemic control is crucial in reducing the incidence of acute and chronic complications, the pressure to find a new hypoglycemic agent is increasing. Currently, nine classes of drugs are available. *Sulfonylureas* are the first oral anti-diabetic drugs used in the treatment of type-2 diabetes. Although long-term use of these drugs is accepted by official guidelines, there are concerns about the side effects of these drugs. The most serious side effects are hypoglycemia and weight gain. Other risks include hepatotoxicity, hematologic dyscrasias, myocardial infarction, allergic reactions and gastrointestinal disorders. Due to the many side effects of antidiabetic drugs, it is necessary to look for alternative agents, such as traditional medicines derived from plants [3]. Traditional medicine has been practiced for thousands of years in Indonesia. It has been used to treat chronic diseases, such as hypertension and diabetes mellitus. Indonesia is a bio-diverse, tropical country with around 25,000 - 30,000 species of plants, accounting for 90% of plant species in Asia. Of these, over 1,000 plants have medicinal properties. One of them is *Persea americana* (Avocado) [4]. *Persea americana Mill*, commonly known as avocado pear, is an evergreen tree belonging to *Lauraceae* family. It is a tropical tree native to Mexico, Central America and South America but has now grown worldwide. *Persea americana* contains high antioxidants, vitamins (*resveratrol* and *vitamin D*), *monounsaturated fatty acids*, *fiber* and *potassium*, which have roles in anticancer, anti-infectious, anti-inflammatory, and prolong life effects [5]. *Persea americana* is used for herbal medicine, especially in several African countries. This plant is believed to have aphrodisiac properties. The fruit skin is used as worm medicine and medicine for dysentery. Extracts from the leaves are used for hypertension and arrhythmias. Grilled and ground leaves and seeds are used for diarrhea and dysentery. Avocado seed oil is used for healing skin diseases. The edible flesh is low in sugar content and thus functions as a high energy source among diabetics [5]. Avocados contain *saponins*, *flavonoids*, *tannins*, *phenols*, *alkaloids*, *steroids* and minerals such as *phosphorus*, *iron*, *potassium*, *magnesium*, and *zinc*. Ethanol extract from avocado seeds contains *polyphenols*, *tannins*, *flavonoids*, *triterpenoids*, *quinones*, *monoterpenoids*, and *sesquiterpenoids*. *Flavonoids* play an important role in the prevention of diabetes and its complications. *Flavonoids* reduce glucose absorption and increase glucose tolerance [6]. The objective of this study was to analyse the hypoglycemic effect of avocado seed extract on male wistar rats. In this study, we randomly grouped 25 rats into five experimental groups with the following treatments: extract 300 mg/kg BW, extract 600 mg/kg BW, extract 1200 mg/kg BW, negative control (aquades distilled water) and positive control (Glybenclamid),

followed by an oral administration of sucrose at 2 mg/kg BW. Blood glucose concentrations were measured and compared among treatment groups.

2. Materials and Method

2.1. Place and time of research

The study was conducted at the Mathematics and Natural Sciences Laboratory of the University of North Sumatra in November 2018

2.2. Research Samples

The sample in this study consisted of avocados purchased from a traditional market in "Pasar Pendidikan" in Medan Timur to obtain 1 kg of dried avocado seeds.

2.3. Tools and Materials

The tools used in this study included: crates, latex gloves, drinking water containers, animal feeds, digital scales, filter paper, ovens, grinders, measuring cups, vacuum evaporators, nasogastric tubes (NGT) no.3 and no.5, 3 mL disposable syringes, syringes 10 cc, 5 cc, 3 cc , scissors, blood sugar measuring device (Gluco Dr, Mediscus, Korea). The materials used in this study include: 96% ethanol, distilled water, glibenclamide, sucrose (granulated sugar) (Rudang jaya, Medan).

2.4. Extraction of Avocado Seeds

The extraction method followed a protocol by Okhiokpamwonyi and his colleagues (2013) [7]. The seeds were separated from the avocado flesh and washed clean. The avocado seeds were then cut and dried in the sun for 5 days. The dried fruit seeds were afterwards mashed with a grinder. Avocado seed flour that has been mashed weighing as much as 645 grams was macerated in 3225 liters of ethanol for 24 hours. After maceration, the solution mixture was filtered. The filtrate was then evaporated in an oven at 30 ° C for 3 days. After that the extract was obtained in the form of brown paste. 40 mg of extract was sent to the Laboratory of Mathematics and Natural Sciences in University of North Sumatra, Medan, for phytochemical analysis. The extract was then stored in a bottle and refrigerated at 4˚C before experiments.

2.5. Experimental Rats

The experimental animals used were white rats, male Wistar strain (*Rattus norvegicus*), aged 8 weeks with body weights of 150-200 grams. Male white rats were obtained from University of North Sumatra that had met these inclusion criteria. 25 Wistar strain male rats were divided into 5 treatment groups, each group consisted of 5 randomly grouped mice. All rats were acclimatized for 7 days to adjust to their environment. The use of experimental animals in this study has been submitted for ethical clearance at an animal development center for North Sumatra research with registration No. 83 / PPHUP / 2019.

2.6. Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) was carried out according to Aom and his colleagues (2017)'s method [8]. The experiments were conducted after rats were fasted for 12 hours and the blood sugar level was measured at fasting. the experimental treatments were given according to the group division, as following:

Group I: aquades distilled water, as a negative control

Group II: 2 mg / kgBW of glibenclamid, as a positive control

Group III: 300 mg / kgBW ethanol extract of avocado seeds

Group IV: 600 mg / kgBW ethanol extract of avocado seeds

Group V: 1200 mg / kgBW ethanol extract of avocado seeds

30 minutes after the above treatments, all groups were given 2 mg / kgBW of glucose. Blood sugar was examined using a glucometer (Gluco Dr, Mediscus, Korea) at -30, 0, 30, 60, 120 and 240 minutes after oral sucrose administration to assess the effect of various experimental treatments on blood glucose levels in rats.

2.7. Statistical Analysis

All statistical analyses were conducted using SPSS. *Shapiro-Wilk* normality tests were used to check for normality in data. Data that were normally distributed (*Shapiro-Wilk*, $p>0.05$), were tested using one-way ANOVA to evaluate the effects of treatments to blood glucose levels at 0 min and 240 mins, followed by *post-hoc* Tukey's HSD multiple comparison test. Data that were not normally distributed (*Shapiro-Wilk*, $p\leq0.05$), were tested using *Kruskal Wallis* test. All significance level in data was set at $\alpha= 0.05$.

3. Result

The phytochemical examination on avocado seed extracts revealed the following contents: *alkaloids*, *tannins*, *saponins*, *steroids*, *flavonoids* and *glycosides*. In this study, blood sugar levels were measured 7 times in all treatment groups: fasting, 30 minutes before treatment, 0 minutes treatment, 30 minutes after treatment, 60 minutes after treatment, 120 minutes after treatment, and 240 minutes after treatment. The means of blood glucose concentration in rats in three extract treatment groups, negative and positive control groups are summarized in Figure 1.

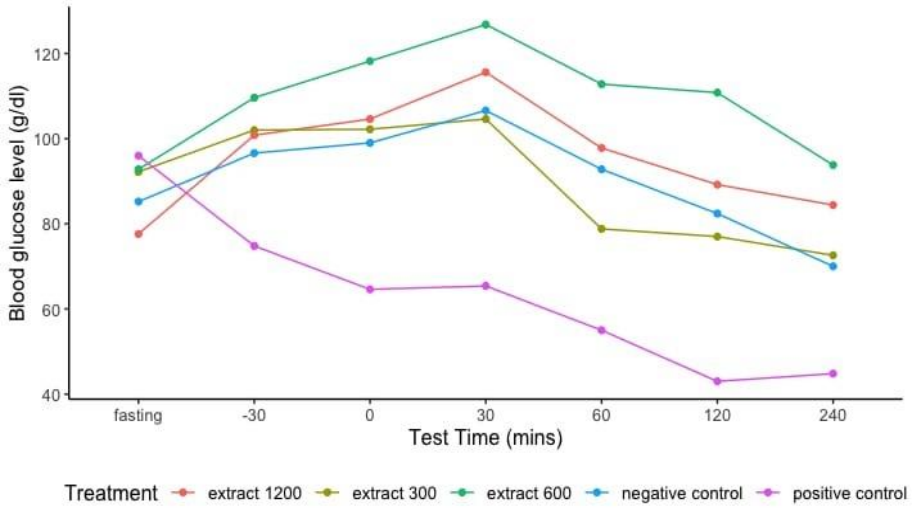

Figure 1: Blood sugar levels (g/dl) following treatments and oral administration of sucrose.

A comparison of the mean differences in blood sugar levels at each measurement time can be seen in Table 1 below.

Table 1: Mean blood sugar level of the whole groups

Time Measurement of Blood Sugar Levels	Mean (mg/dl)	SD	Mean Rank	p-value
fasting	88.76	9.5	3.40	<0.05
30 minutes before treatment	96.76	15.2	4.80	
0 minutes	97.72	20.4	5.12	
30 minutes after treatment	103.8	22.8	6.20	
60 minutes after treatment	87.44	21.9	3.80	
120 minutes after treatment	80.48	24.4	2.66	
240 minutes after treatment	73.12	20.25	2.02	

The differences in the mean measurement of blood sugar levels were the highest at 30 minutes after treatment (103.8 mg/dl) and lowest at 240 minutes after treatment (73.12 mg/dl). A comparison between the mean differences in blood sugar levels at 0 minute and at 240 minutes between test groups can be seen in Table 2 below.

Table 2: Mean blood sugar level according to the treatment group

Test group	Mean (mg/dl)	SD	95% CI		p-value
			Lower	Upper	
Negative Control	-26.80	17.8	-48.86	-4.74	>0.05
Positive Control	-23.20	18.7	-46.45	0.05	
Avocado seed extract 300mg / kgBB	-29.39	25.8	-61.65	2.45	
Avocado seed extract 600mg / kgBB	-24.40	6.5	-32.48	-16.32	
Avocado seed extract 1200mg / kgBB	-20.20	13.1	-36.52	-3.88	

The mean decrease in blood sugar levels was the highest in the avocado seed extract group at 300mg / kgBB (-29.39 gr / dl) and the lowest was found in the avocado seed extract group at 1200mg / kgBB (-20.20 gr / dl). All test groups showed decreases in blood sugar levels when comparing blood sugar levels at 0 minute of treatment with 240 minutes of treatment. Data for measurement of the average decrease in blood sugar levels were normally distributed (*Shapiro-Wilk*, $p > 0.05$). There were no significant differences in the mean reduction in blood sugar levels among the test groups (one-way ANOVA, $p > 0.05$).

4. Discussion

From the research results above, it was found that there was a decrease in blood glucose levels in rats in the treatment using avocado seed extract at all extract concentrations. This is consistent with research conducted by Oktaria and his colleagues (2015) on the effects of ethanol extract of avocado seeds at300; 600; 1200 mg / KgBW, which reduced blood glucose levels in alloxan-induced mice [6]. The mean decreases in blood sugar levels were not significant when compared to the positive control group. This is in accordance with the study of Msudja and his colleagues (2017) who examined the effects of guajava (*Psidium guajava L*) leaf extract which is known to have flavonoid content with hypoglycemic activity but the effect was not significant when compared to the control group [9]. A similar result was found in a study conducted by Aom and his colleagues (2017) which examined the extract of *Phyllantus Acidus L* containing flavonoid compounds by OGTT examination showing hypoglycemic activity in experimental animals but the effect was not significant when compared with the control group [8]. This is probably because the way this research works is related to stimulating insulin which can increase glucose transport through cell membranes into cells. The speed of glucose transport through the cell membrane is strongly influenced by the amount of insulin secretion. When insulin is released in a large amount by beta pancreatic cells, the transport of glucose through the cell membrane can be 10 times faster than that without insulin secretion [10]. The regulation of insulin works when food rich in carbohydrates enters the digestive system and activates two primary hormones, insulin and glucagon. The digestive system will break down carbohydrates into smaller molecules called *monosaccharides* which are then absorbed through glucose transporters (GLUT) into blood vessels. GLUT is derived from the SLC2-encoded gene and is responsible for the transport of *monosaccharides*, polysaccharides and other small components through cell membranes. There are fourteen GLUT proteins present in humans, namely GLUT 1-12, GLUT 14 and *myoisositol* transporter (HMIT). GLUT 2 is responsible for transporting glucose from circulation to pancreatic cells to stimulate insulin release. Decreased blood sugar levels have several mechanisms, namely 1. Increased uptake of glucose in peripheral tissue through translocation of GLUT 4; 2. Inhibits lipolysis and increases lipogenesis; 3. Increase glucose storage and glucose usage by the liver. Conversely, when glucose levels in the blood are low then the release of glucagon hormone will increase. Increased glucagon will lead to 1. Increased breakdown of glucose in the liver; 2. Increase lipolysis so that it breaks down fat deposits in the body [11]. The concentration of insulin in plasma is easily changed due to the physiology of insulin release from pancreas. Insulin is released into plasma pulsatively according to changes in its concentration in blood. A balance in the concentration of insulin in blood serves to prevent insulin resistance in cell membrane receptors. Loss of insulin control in the blood is an early indication of diabetes [12]. Chemical substances that are suspected to have the effect of reducing blood glucose are *flavonoids* and *saponins*. *Flavonoids* are a class of secondary metabolites of plants with flavone (2-phenylchrome-4-one) bonds. The most important subgroups of

flavones are *flavonols, flavones, isoflavones, flavanones, flavanonols* and *anthycyanins*, which are differentiated based on the structure of chemical bonds. The *flavonoid* content has biological activity and has been investigated for its potential as a therapy for diabetes and its complications [13]. How*flavonoids* worksto reduce blood sugar levels in mice is the antioxidant effect that provides protection to pancreas against oxidative stress. While the way *saponins* works is by suppressing glycolysis and glycogenesis in the liver. Tiwari & rao revealed that *flavonoids, tannins*, and *saponins* have hypoglycemic effects by inhibiting the action of sodium glucose transporter 1 (S-GLUT 1) [6]. Flavonoids from all sub-classes have been shown to have hypoglycemic properties by increasing insulin secretion through regeneration of pancreatic β cells, increasing glucose uptake that is mediated by insulin by target cells, inhibiting aldosterone reductase and increasing calcium absorption. Flavonoids have powerful antioxidants that can reduce blood glucose levels in the treatment of diabetic patients. The effect of hypoglycemia is caused by the chemical form of flavonoids, namely double bonds [(C-2- C-3) and the presence of ketonic groups in C-4 that are in B bonds] which are fundamentally bioactive of poly-phenol content. These chemicals play an important role in maintaining blood glucose levels, glucose use, insulin secretion and immunomodulatory functions to prevent diabetes mellitus [14].

5. Conclusion

It can be concluded that avocado seed extract with oral glucose tolerance test (OGTT) can reduce blood glucose levels but not significantly ($p > 0.05$) when compared with glibenclamide administration as a positive control. The highest decrease in blood sugar level was observed following the administration of avocado seed extract at a dose of 300 mg / kgBW.

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