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Research Article
Effect of Avocado Leaf Extract on the Decrease of Fasting Blood Glucose Level of White Rats

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Abstract

Background and Objective: Preclinical testing of the use of avocado leaf extract for decreasing blood glucose level is still limited. This study aimed to analyze the effect of avocado leaf extract on fasting blood glucose level in male white rats. **Materials and Methods:** This study was a pre-experiment with six groups of pretest posttest with control group. The samples were male white rats (*Rattus norvegicus*) of Wistar strain, ±3 months old, with the treatment groups: Group 1: Feed+Streptozotocin (STZ)+10% sucrose+avocado leaf extract dose of 100 mg kg^{-1} b.wt., Group 2: Feed+STZ+10% sucrose+avocado leaf extract dose 150 mg kg^{-1} b.wt., Group 3: Feed+STZ+10% sucrose+avocado leaf extract dose 200 mg kg^{-1} b.wt., Group 4: Feed+STZ+10% sucrose, Group 5: Feed+STZ+10% sucrose+glibenclamide and Group 6: Feed. Data analysis used ANOVA and paired t-test with $p<0.05$. **Results:** The early fasting blood glucose level of the white rats was 58-97 mg dL^{-1} ($p = 0.897$). Giving STZ increases the fasting blood glucose level to 83-128 mg dL^{-1} ($p = 0.136$). Meanwhile, giving avocado leaf extract on day 14 decreased 68-77 mg dL^{-1} ($p = 0.000$). Group 2 experienced a significant decrease on day 7, which was 28.22 mg dL^{-1} ($p = 0.028$) and day 14, which was 31.33 mg dL^{-1} ($p = 0.015$). **Conclusion:** Avocado leaf extract with a dose of 150 mg kg^{-1} b.wt. is effective in reducing the high fasting blood glucose level in white rats.

Key words: Avocado leaf extract, fasting blood glucose, white rats, streptozotocin, glibenclamide

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Uncontrolled glucose level is the main problem experienced by patients with Type 2 Diabetes Mellitus (T2DM) which can cause complications such as heart disease, hypertension, stroke, kidney failure, blindness, cancer and impotence[1,2]. People generally use certain parts of plants to reduce high blood pressure, blood glucose level and cholesterol. The use of plant parts raises questions related to the quality, efficacy and safety[3]. Therefore, it needs to be empirically investigated. Most natural anti-oxidants come from fruits, vegetables, spices and grains such as ginseng, curcuma, rosemary, green tea, grapes, ginger, garlic and avocado[4-7].

Previous studies showed that plant leaf extract could reduce blood glucose level in white rats, such as Sintowati *et al.*[8] that used 70% methanol extract of avocado leaves (*Persea americana* Mill) with a dose of 200 mg/200, 100 mg/200 and 150 mg/200 g/body weight which can reduce blood glucose level in male Wistar white rats. Furthermore, Putri *et al.*[6] found that avocado leaf extract is able to decrease the level of glucose and the most effective concentrate is 10%. Harianto's research on the effect of n-butanol fraction of avocado leaves (*Persea americana* Mill) extract on male white rats showed that it can reduce blood glucose level effectively at a dose[9] of 1.0 g kg^{-1} b.wt.

Other studies showed that avocado leaf extract using ethanol solution displays a strong antioxidant activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) and that the effects of phytochemical combinations (flavonoids, saponins, tannins and steroids) from avocado leaf extract can be used in the treatment of oxidative stress[10].

Based on the previous research, this study was conducted by using different dose variants and avocado leaf extract by using ethanol solution to produce avocado leaf extract products that are useful for lowering blood glucose level. Preclinical testing of avocado leaf extract is needed to assess the ability of avocado leaves in reducing blood glucose level. Therefore, this study aimed to analyze the effect of avocado leaf extract on fasting blood glucose level in male white rats.

MATERIALS AND METHODS

Research type: This study was a pre-experiment with six groups of pre-test and post-test with control group. This research was carried out in the Pharmacology Laboratory of Pelita Mas Palu STIFA for 5 months in 2018.

Preparation of test animals: Eighteen male Wistar strain white rats were adapted for 2 weeks in a laboratory with adequate cages at normal ambient temperatures and given standard feed and drinking. The type of standard feed was Turbo Feed with a protein of 16-18%, fat 4%, ash content 12%, fiber 8% and moisture content 12%.

Tools: Glucose measuring devices (Accu Check Active), Aluminum Foil, Stirring Rods, Blenders (Panasonic), Porcelain Cups, Funnels, 1000 mL Chemical Glasses (Agc Iwaki Cte 33), 100 mL Measuring Cups (Pyrex), Test Animal Enclosures, Measuring Flasks 100 Ml, Mortar and Stamper, Pipette Drops, Rotary Evaporator (Heidolph), Oral Sonde, Injection Spoit (Treumo) 3 and 5 mL, Glucose Strips, Reaction Tubes, Analytical Scales (Ohaus), Gram Scales, Water bath and Maceration Containers.

Avocado leaf extract: Avocado leaves were extracted with 70% of positive ethanol solution containing flavonoid compounds, saponins, tannins, steroids. The 70% concentration is an avocado leaf extract that gives absorbance value and optimum percent inhibition[10].

Testing of antidiabetic effects: On day 0 after adaptation, the rats were fasted for 16 h. Then, the initial fasting blood glucose level was measured. After the initial blood glucose level was measured, on the same day, streptozotocin (STZ) rats were induced with 30 mg kg^{-1} b.wt., intraperitoneally. On the 3rd day after the induction, the rats were fasted for 16 h. Then, the blood glucose level of the rats was measured. After the fasting blood glucose level of the rats had reached a state of hyperglycemia, they were given the oral treatment for 7 days. The treatment was continued up to 14 days.

Treatment groups:

Group 1 : Feed+STZ+10% sucrose+avocado leaf extract dose of 100 g kg^{-1} b.wt.
Group 2 : Feed+STZ+10% sucrose+avocado leaf extract dose of 150 g kg^{-1} b.wt.
Group 3 : Feed+STZ+10% sucrose+avocado leaf extract dose 200 g kg^{-1} b.wt.
Group 4 : 10% feed+STZ+sucrose
Group 5 : Feed+STZ+10% sucrose+glibenclamide
Group 6 : Feed

Time for measurement of body weight and fasting blood glucose level:

Measurement 1 : Before STZ induction
Measurement 2 : After STZ induction
Measurement 3 : 7 days after the intervention of avocado leaf extract
Measurement 4 : 14 days after the intervention of avocado leaf extract

Research ethics: This study has obtained an ethical recommendation agreement Number: 546/H4.8.4.31/PP36-KOMETIK/2018 issued by the Health Research Ethics Committee of the Faculty of Medicine RSPTN Hasanuddin University, RSUP Dr. Wahidin Sudirohusodo Makassar.

Data analysis: Data analysis was conducted by using one-way ANOVA test and paired t-test with a significance level of 5% or $p<0.05$.

RESULTS

As Table 1 showed the white rats were grouped into 6 groups at the beginning of the study with 18 white rats not having a significantly different body weight with p-value = 0.090 ($p>0.05$). The body weight of the white rats ranged from 133-225 g. Before the intervention of avocado leaf extract was done, an attempt to increase the fasting blood glucose level was done by giving STZ to the white rats. ANOVA test results showed that there were no significant differences in body weight among the 6 groups after the rats were given STZ with $p = 0.095$ ($p>0.05$).

On the 7th day after the rats were given avocado leaf extract, the body weight showed a significant difference between the 6 groups with $p = 0.016$ ($p<0.05$). Rats in groups 3, 5 and 6 had the heaviest body weight of 239.33 g (groups 3), 240.00 g (groups 5), 249,67 g (groups 6) (Table 1). Measurement IV is 14 days after the intervention of avocado leaf extract necessary to find out the effective time of avocado leaf extract on weight and fasting blood glucose of mice.

As Table 2 showed the fasting blood glucose level of white rats grouped into 6 groups was not different with $p = 0.897$ ($p>0.05$) and the fasting blood glucose level of the white rats ranged from 58-97 mg dL^{-1}. The giving of STZ at the second measurement also showed that there were no significant differences in fasting blood glucose level between the 6 groups with $p = 0.136$ ($p>0.05$) and fasting blood glucose level of the white rats ranged from 83-128 mg dL^{-1}.

The fasting blood glucose level of the white rats on day 14 after the rats had been given the avocado leaf extract showed a significant difference between 6 groups with $p = 0.000$ ($p <0.05$). Group 2 had the lowest fasting blood glucose level of 73.33±4.72 mg dL^{-1} and group 4 had the highest fasting blood glucose level of 104.00±10.81 mg dL^{-1} (Table 2).

Table 1: White rats body weight during research

Measurement time	Treatment groups	n	Body weight (g) Mean±SD	Min-max	p-value (one-way ANOVA test)
I	Group 1	3	151.67±16.28	133-163	0.090
	Group 2	3	202.67±28.92	170-225	
	Group 3	3	185.67±17.21	166-198	
	Group 4	3	187.67±32.32	152-215	
	Group 5	3	209.33±9.02	200-218	
	Group 6	3	210.33±31.34	182-244	
II	Group 1	3	158.00±22.11	133-175	0.095
	Group 2	3	208.67±26.50	179-230	
	Group 3	3	199.67±11.02	189-211	
	Group 4	3	189.33±29.28	158-216	
	Group 5	3	210.00±13.00	202-225	
	Group 6	3	214.00±29.54	190-247	
III	Group 1	3	178.00±20.95	155-196	0.016
	Group 2	3	213.33±23.24	187-231	
	Group 3	3	239.33±10.21	232-251	
	Group 4	3	195.67±24.13	169-216	
	Group 5	3	240.00±15.58	231-258	
	Group 6	3	249.67±36.67	221-291	
IV	Group 1	3	194.33±17.15	176-210	0.078
	Group 2	3	224.67±19.29	203-240	
	Group 3	3	232.67±10.97	220-239	
	Group 4	3	214.33±19.14	193-230	
	Group 5	3	247.00±11.35	239-260	
	Group 6	3	246.67±38.83	215-290	

Table 2: White rats fasting blood glucose level during research

Measurement time	Treatment groups	n	Fasting blood glucose level (mg dL^{-1}) Mean±SD	Min-max	p-value (one-way ANOVA test)
I	Group 1	3	82.00±8.88	72-89	0.897
	Group 2	3	73.00±13.22	58-83	
	Group 3	3	78.00±6.08	71-82	
	Group 4	3	77.67±5.85	71-82	
	Group 5	3	72.00±12.00	60-84	
	Group 6	3	78.67±18.50	60-97	
II	Group 1	3	114.67±13.01	102-128	0.136
	Group 2	3	104.67±2.08	103-107	
	Group 3	3	98.00±14.52	83-112	
	Group 4	3	93.67±8.96	88-104	
	Group 5	3	104.67±2.30	102-106	
	Group 6	3	97.00±4.35	92-100	
III	Group 1	3	90.67±8.50	81-97	0.321
	Group 2	3	76.33±6.42	69-81	
	Group 3	3	169.00±111.90	98-298	
	Group 4	3	93.67±18.14	77-113	
	Group 5	3	129.67±57.81	90-196	
	Group 6	3	91.67±4.04	87-94	
IV	Group 1	3	93.67±5.68	89-100	0.000
	Group 2	3	73.33±4.72	68-77	
	Group 3	3	76.00±5.29	70-80	
	Group 4	3	104.00±10.81	95-116	
	Group 5	3	91.33±2.08	89-93	
	Group 6	3	82.33±1.15	81-83	

Table 3: Fasting blood glucose (FBG) level after giving avocado leaf extract test

Treatment groups	n	Δ FBG(I) vs. FBG(II)	p-value	Δ FBG(II) vs. FBG(III)	p-value	Δ FBG(II) vs. FBG(IV)	p-value
Group 1	3	32.66	0.111	24.00	0.096	21.00	0.147
Group 2	3	31.66	0.048	28.22	0.028	31.33	0.015
Group 3	3	20.00	0.063	71.00	0.342	22.00	0.190
Group 4	3	16.00	0.201	0.00	NA	10.33	0.042
Group 5	3	32.66	0.044	25.00	0.546	13.33	0.022
Group 6	3	18.33	0.172	5.33	0.004	14.66	0.036

Paired t-test, FBG(I): Fasting blood glucose before STZ induction, FBG(II): Fasting blood glucose after STZ induction, FBG(III): Fasting blood glucose 7 days after the intervention of avocado leaf extract, FBG(IV): Fasting blood glucose 14 days after the intervention of avocado leaf extract, NA: Not acceptable, Δ: Delta

Table 3 showed that there were significant differences in fasting blood glucose level after the rats in group 2 (difference of 31.66 mg dL^{-1}, $p = 0.048$) and group 5 (difference of 32.66 mg dL^{-1}, $p = 0.044$) were given STZ. This showed that fasting blood glucose for group 2 and 5 increased after STZ induction. The difference in fasting blood glucose level after giving avocado leaf extract for 7 days showed that group 2 and group 6 experienced a significant decrease with $p<0.05$.

The significant difference in fasting blood glucose level after the giving of avocado leaf extract for 14 days showed that groups 2, 5 and 6 experienced a significant decrease with $p<0.05$, while group 4 experienced a significant increase with $p = 0.042$.

DISCUSSION

The results showed that avocado leaf extract had a significant effect in reducing fasting blood glucose level in white rats at a dose of 150 g kg^{-1} b.wt. The dose of 100 and 200 g kg^{-1} b.wt., did not show a positive change in fasting blood glucose level in white rats. This indicated that the dose of avocado leaf extract that is too low and high does not have a positive effect on decreasing fasting blood glucose level.

The white rats used in this study had normal fasting blood glucose level as shown in the first measurement in Table 2. Furthermore, Streptozotocin (STZ) injection was done in group 1-6 aimed to increase fasting blood glucose level in white rats. This is because STZ injected intraperitoneally acts as diabetogenic which will enter pancreatic β cells through the glucose transporter (GLUT-2)[11]. This study also used glibenclamide in group 5 as a positive control. This was used as a comparison of the effects of avocado leaf extract with various doses[12]. This study showed that a dose of 150 mg dL^{-1} equals the ability of glibenclamide to reduce fasting blood glucose level. This might be due to the activity of extracts of natural ingredients which are multicomponent mixtures so that the effects of these components can be synergistic, additive or antagonistic[13,14].

The results of other studies showed that a dose of 150 mg kg^{-1} b.wt. had the effect of lowering fasting blood glucose level in white rats, better than the dose[8] of 100 mg kg^{-1} b.wt.. Research conducted by Sintowati *et al.*[8] also showed that the higher doses of avocado leaf extract can reduce the fasting blood glucose level better than the low doses. Other studies have shown that avocado leaf extract with 1.960 kg b.wt., can reduce the glucose of 64.27% and there is no significant difference compared to the positive control glipizid[15] is 68.50%.

The decrease in blood glucose level by giving avocado leaf extract showed that the anti-oxidant content of avocado leaf extracts such as flavonoids and tannins had the effect of lowering blood glucose level in test animals. Qualitative analysis of phytochemical compounds on avocado leaf extract shows that it contains flavonoid compounds, saponins, tannins, triterpenoids, steroids and no alkaloids[10]. Flavonoids contained in avocado leaves (*Persea americana* Mill) acts as hypoglycemic agents[16]. Saponins work by inhibiting the action of the enzyme α-glucosidase, an enzyme in the intestine which functions to convert carbohydrates into glucose[17]. The role of tannins is to capture free radicals and reduce increased oxidative stress in diabetics to control blood glucose level[18].

The implication of this study is that avocado leaf extract can be used to reduce fasting blood glucose level with the recommended dose of 150 mg kg^{-1} b.wt. This study is a preclinical test. Therefore, further research on humans is needed to get a positive effect of the extract on humans, especially patients with high fasting blood glucose level. In terms of research limitations, which anti-oxidant compounds in avocado leaves that have the ability as anti-diabetic to reduce blood glucose level has not been known.

CONCLUSION

Avocado leaf extract can reduce high fasting blood glucose level in test animals after intervention for 7 days and 14 days at a dose of 150 mg kg^{-1} b.wt. This preclinical test shows that dosing of avocado leaf extract that is too low and high does not have a positive effect in reducing fasting blood glucose level.

SIGNIFICANCE STATEMENT

This study reveals that avocado leaf extract with its phytochemical content and antioxidant activity can reduce fasting blood glucose level in white rats. This study will help researchers determine the right dose of avocado leaf extract significantly reduces the fasting blood glucose level in white rats. This suggests that a high dose of avocado leaf extract in white rats does not significantly decrease the fasting blood glucose level.

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