Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Mar 11; 10(A):415-418.
https://doi.org/10.3889/oamjms.2022.8582
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Effect of Avocado (*Persea Americana Mill.*) Peel Extract on the Diabetic Male White Rats: Preclinical Study

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Edited by: Sinisa Stojanoski
Citation: Rahman N, Tangkas IM, Rakhman A, Sabang SM, Bohari B. Effect of Avocado (*Persea Americana Mill.*) Peel Extract on the Diabetic Male White Rats: Preclinical Study. Open Access Maced J MedSci.2022Mar11;10(A):415-418.https://doi.org/10.3889/oamjms.2022.8582
Keywords: Fasting blood glucose level; Avocado peel extract; Streptozotocin; Mice
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Received: 10-Jan-2022
Revised: 19-Feb-2022
Accepted: 10-Mar-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Type 2 Diabetes mellitus (DMT2) is one of the most common non-communicable diseases (NCDs) worldwide. T2DM is also the leading cause of death in most developed and developing countries.

AIM: This study aimed to analyze the effect of avocado (*Persea Americana Mill.*) skin extract on blood glucose levels in white male rats with diabetes (preclinical study).

METHOD: Experimental research *in vivo* with pre-test and post-test only control group design. The samples were 15 white male rats aged ± 3 months, with a 150–200 g bodyweight. Avocado peel was extracted with ethanol. Measurement of fasting blood glucose levels was carried out 3 times. Streptozotocin administration was used to increase glucose levels in experimental animals. Rats were induced by streptozotocin with 30 mg/kg BW intraperitoneally on the same day. Data analysis used Analysis of Variance (ANOVA) to analyze the data obtained from each treatment group, and the level of significance was expressed in = 5%.

RESULTS: Fasting blood glucose levels in the first measurement for all groups were included in the normal category, about 48.73 mg/dL. Then, the second measurement after being induced with STZ showed an increase in fasting blood glucose levels of mice with an average of 181.07 mg/dL. The third measurement showed that mice's fasting blood glucose level was still high, except for Group IV, which decreased to 97.33 mg/dL. The results of the comparative analysis of fasting blood glucose levels on the second and third measurements showed that most of them experienced a decrease, except in Group V, and the intervention group which experienced a significant decrease, Group IV with p = 0.003 and the magnitude of the decrease was 133.33 mg/dL.

CONCLUSION: The concentration of 200 mg/kg BW of avocado peel extract significantly reduced fasting blood glucose levels by 133.33 mg/dL after STZ induction compared to other groups.

Introduction

One problem of non-communicable diseases that are common and cause death is diabetes mellitus, especially diabetes mellitus type 2 [1]. T2DM has become one of the most influential public health problems in the 21st century. The prevalence of T2DM in Central Sulawesi based on diagnosis and symptoms is 3.7% among adults and shows that it is the province with the highest prevalence of T2DM in Sulawesi Island [2].

Patients with DMT2 are prone to experience the formation of excess free radicals, which can later cause damage to the pancreas. This can interfere with pancreatic beta-cell function and insulin resistance to worsen diabetes conditions [3], [4]. These harmful free radicals require an antioxidant to counteract them. One of them is relatively cheap alternative medicines, and the efficacy is not much different from synthetic drugs. However, research on the type of phytochemical content, preclinical, and clinical trials of avocado leaves to prevent this disease is still relatively limited.

Avocado plant parts, especially avocado flesh, have significantly lower blood glucose. Avocado flesh and seeds contain saponins, alkaloids, flavonoids, and tannins [5], [6]. Meanwhile, avocado seeds were also reported to effectively reduce blood glucose levels of rats that had been induced with alloxan [7], [8]. The results of previous studies on avocado leaves showed that avocado leaves contain phytochemicals and have strong antioxidant power against DPPH [9]. However, waste from avocado fruit (avocado skin) can also be used. Its chemical content is more important, namely, flavonoids, because it is one of the largest natural phenolic compounds found in all green plants. Then, researchers are interested in researching the skin of the avocado.

This study aimed to determine the effect of avocado peel extract (*Persea Americana Mill.*) on blood glucose levels in white male rats with diabetes (preclinical study).

Methods

This research was experimental *in vivo*. The design used was true experimental – pre-test and post-test only control group design. The population of this study was 15 white male rats of the Wistar strain (*Rattus norvegicus*), aged ± 3 months, with a bodyweight of 150–200 g. Avocado peel (*Persea Americana Mill.*) extracted with ethanol, white male rats obtained from Malang, distilled water, 70% alcohol, citrate buffer, 96% ethanol, cotton, filter paper, Na CMC, standard feed, and streptozotocin. Glucose Measuring Instrument (Easy Touch®GCHb), Aluminum Foil, Stirring Rod, Blender (Panasonic), Porcelain Cup, Funnel, 1,000 Ml Beaker (AGC Iwaki Cte 33), 100 Ml Measuring Cup (Pyrex), Test Animal Cage, Pumpkin Measure 100 Ml, Mortar and Stamper, Dropper Dropper, Rotary Evaporator (Heidolph), Oral Sonde, Injection Spray (Treumo) 3 Ml And 5 Ml, Glucose Strip, Test Tube, Analytical Scale (Ohaus), Coarse Gram Scale, and Waterbath and Container Maceration were used.

On day 0 after adaptation, the rats fasted for 16 h, then, the initial blood glucose levels were measured. After measuring initial blood glucose levels, rats were induced by streptozotocin with 30 mg/kg BW intraperitoneally on the same day. The 3rd day after induction, the rats were fasted for 16 h and then re-measured the rats' blood glucose levels after induction. After the fasting blood glucose levels of the rats had reached a hyperglycemic state (> 142 mg/dL), they were given oral treatment for 14 days. The long consideration of giving avocado leaf extract for 14 days is based on previous research using the treatment that was carried out for 14 days and observations made on blood glucose levels and body weight of mice [10].

The treatments given were as follows: (1) Group 1 only received feed; (2) Group 2 received Feed + STZ + 10% Sucrose + No Avocado Peel Extract; (3) Group 3 received feed + Streptozotocin + 10% sucrose + avocado peel extract at a dose of 100 ml/kg BW; (4) Group 4 received feed + Streptozotocin + 10% sucrose + avocado leaf extract at a dose of 200 ml/kg BW; and (5) Group 5 received Feed + Streptozotocin + 10% Sucrose + Avocado Peel Extract Dosage 400 ml/kg BW.

The results obtained from the study were analyzed using the analysis of variance (ANOVA), which was conducted to analyze the data obtained from each treatment group. The level of significance was expressed in = 5%.

Results

Table 1 shows that fasting blood glucose levels in the first measurement for all groups were in the normal category, about 48.73 mg/dL. Then, the second measurement after being induced with STZ showed an increase in fasting blood glucose levels of mice with an average of 181.07 mg/dL. The third measurement showed that mice's fasting blood glucose level was still high, except for Group IV, which decreased to 97.33 mg/dL. The results showed that the highest average fasting blood glucose level at week 3 of FBG measurement, which was 497.67 mg/dL, and groups 3 and 4 experienced a decrease in FBG levels during the administration of avocado peel extract of 100 ml/kg BW and 200 ml/kg BW from 191 mg/dL to 166.33 mg/dL (Group 3) and 230.67 mg/dL to 166.33 mg/dL (Group 4).

Table 1: Distribution of fasting blood glucose levels (mg/dL) in mice according to intervention group

Fasting blood glucose	N	Mean	Standard Deviation	Minimum	Maximum
FBG 1					
I	3	47.00	6.08	43.00	54.00
II	3	50.67	5.13	45.00	55.00
III	3	48.33	8.50	42.00	58.00
IV	3	44.67	4.04	40.00	47.00
V	3	53.00	11.14	43.00	65.00
Total	15	48.73	6.95	40.00	65.00
FBG 2					
I	3	112.67	34.85	81.00	150.00
II	3	204.33	49.54	150.00	247.00
III	3	191.00	31.80	162.00	225.00
IV	3	230.67	6.43	226.00	238.00
V	3	166.67	24.70	144.00	193.00
Total	15	181.07	49.73	81.00	247.00
FBG 3					
I	3	78.67	20.65	55.00	93.00
II	3	197.00	167.31	93.00	390.00
III	3	160.67	84.67	104.00	258.00
IV	3	97.33	6.11	92.00	104.00
V	3	497.67	90.26	400.00	578.00
Total	15	206.27	175.91	55.00	578.00

Table 2 shows that there was no difference in the fasting blood glucose levels of mice in the first measurement in five groups with p = 0.69 (p > 0.05). The second measurement showed that the average fasting blood glucose level of mice increased after induction, obtained from the one-way ANOVA test between the control group and the intervention group showed a significant value with p = 0.01. Then, in the third measurement, after the intervention, as giving avocado skin extract with various concentrations, it showed a significant difference with p = 0.00. The results of the comparative analysis of fasting blood glucose levels on the second and third measurements showed that most of them experienced a decrease except in Group V and the intervention group, which experienced a significant decrease, in Group IV with p = 0.003, and the magnitude of the decrease was 133.33 mg/dL.

Table 2: Average fasting blood glucose in mice (mg/dL) in measurements 1, 2, and 3

Treatment	N	The average blood glucose levels (mg/dL) of male mice			FBG 3 vs. FBG 2	
		FBG 1	FBG 2	FBG 3	Δ	p
I	3	47.00 ± 6.08	112.67 ± 34.85	78.67 ± 20.65	–34.00	0.397
II	3	50.67 ± 5.13	204.33 ± 49.54	197.00 ± 167.31	–7.33	0.958
III	3	48.33 ± 8.50	191.00 ± 31.80	160.67 ± 84.67	–30.33	0.624
IV	3	44.67 ± 4.04	230.67 ± 6.43	97.33 ± 6.11	–133.33	0.003
V	3	53.00 ± 11.14	166.67 ± 24.70	497.67 ± 90.26	331.00	0.035
p (One-Way Anova)		0.69	0.01	0.00		

Discussion

The results showed that Group 4, with the intervention of 200 mg/kg BW of avocado peel extract, experienced a significant decrease in fasting blood glucose levels of 133.33 mg/dL after STZ induction compared to other groups. Streptozotocin (STZ) is a diabetogenic agent used as an experimental animal model of diabetes. STZ inhibits insulin secretion and causes pancreatic beta-cell necrosis and increased blood glucose levels [11]. In this study, three groups received avocado peel extract with different concentrations, and the results showed that the group with a concentration of 200 ml/kg BW had better results and was able to significantly reduce fasting blood glucose levels compared to a high concentration of 400 mg/kg BW.

The results of this study are in line with previous studies, the administration of avocado leaf extract with a concentration of 10% is very effective in lowering blood glucose in mice. The effectiveness of avocado leaf extract in lowering blood glucose may be influenced by the bioactive substances contained in the avocado leaf extract. The bioactive substances contained in the extract more affected the decrease in blood glucose in the avocado leaf extract with a concentration of 10% compared to 20%, 40%, and the drug glibenclamide [12]. Here, it can be seen that the effect is smaller than the smallest concentration at the largest concentration used. This is often found in extracts of natural ingredients, which are multicomponent mixtures. The effects of these components can be synergistic, additive, or antagonistic. The possibility of higher concentrations of avocado leaf extract exacerbating insulin-producing tissue damage cannot be ruled out.

The results of other studies showed that the level of leaf aging and drying method affected the bioactive compounds and antioxidant activity contained in the avocado leaf extract produced. Oven-dried old leaves produced the highest antioxidant activity with specific air content, total phenol, total flavonoid, and total tannin [13]. Avocado peel extract contains very high phenolic compounds and has good antioxidant activity. The content of phenolic compounds in avocado skin is Procyanidin dimer A, Quercetin glucoside, Quercetin 3-O-arabinose-glucoside, Procyanidin trimer B-isomer 1, Procyanidin trimer B-isomer 2, Procyanidin dimer B-isomer 3, Procyanidin trimer A, Procyanidin dimer B2, Procyanidin trimer B-isomer 3, Quercetin 3-O-arabinoside, and Quercetin 3-O-rhamnoside [14]. Phenolics are part of polyphenolic compounds that function as antidiabetics [15]. Phenol compounds act as antioxidants directly by donating hydrogen ions to stabilize free radicals and act as direct free radical scavengers [16], [17].

Then, the antioxidant content found in the leaves and skin of avocados can inhibit oxidative stress in patients with type 2 diabetes. Antioxidants are electron-donating compounds that inhibit oxidation reactions by binding to free radicals and highly reactive molecules to prevent cell damage [18]. Prevention efforts to increase oxidative stress and decrease antioxidant activity in the body consume foods that are sources of antioxidants [19]. The effect of flavonoid administration in preventing oxidative stress in streptozotocin-induced diabetic rats showed that administration of quercetin (a flavonoid) at a dose of 15 mg/kg caused a significant decrease in the increase in malondialdehyde (MDA) and nitric oxide (NO) and an increase in enzymatic antioxidant activity [18].

The results of this study are in line with the research of Iskandar *et al*. (2019), which showed that drinking powder was able to reduce blood glucose levels in hyperglycemic mice significantly, and the best treatment was giving avocado seed drink powder at a dose of 540 mg/kg BW which could reduce blood glucose levels by 44% and weight loss, body weight by 27.32% [20]. Another study about the combination of papaya seed and avocado seed infusion with concentrations of 0.4% v/v, 0.6% v/v, and 0.8% v/v, lowered blood glucose levels, and the most effective concentration in lowering blood glucose level is 0.6% v/v [21].

Conclusion

The concentration of 200 mg/kg BW of avocado peel extracts significantly reduced fasting blood glucose levels by 133.33 mg/dL after STZ induction compared to other groups. Concentrations that were too low and high in avocado peel extract did not show a significant and positive change in improving fasting blood glucose levels in mice.

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