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Comparison of Effectiveness of Milling Time of Avocado Seed Extract for Lowering Blood Glucose Level in Alloxan-induced Diabetic Rat

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Abstract

Introduction:Diabetes Mellitus (DM) is a chronic metabolic disorder with increasing global prevalence. Effective management is crucial to prevent acute and long-term complications. Natural products, including avocado (*Persea americana* Mill.) seeds, have gained attention for their potential antidiabetic properties due to their rich phytochemical content.

Methods: This experimental study used male Sprague Dawley rats, divided into two main groups based on blood glucose status (normal and diabetic), with each group further subdivided based on the milling duration of avocado seed extract (5 and 10 minutes). The extract was administered orally at a dose of 250 mg/kg body weight for seven consecutive days. Blood glucose levels were measured using a glucometer before and after the treatment period.

Results: Avocado seed extract demonstrated a glucose-lowering effect in both treatment groups. Rats treated with extract milled for 5 minutes showed a mean reduction in blood glucose of 25.13 mg/dL, while those receiving extract milled for 10 minutes exhibited a greater reduction of 45.88 mg/dL. Although the group treated with the 10-minute extract showed a higher decrease, statistical analysis using the Mann–Whitney U test revealed no significant difference in glucose-lowering efficacy between the two milling durations ($P > 0.05$).

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Conclusions:Avocado seed extract, regardless of milling duration, has potential antihyperglycemic effects in alloxan-induced diabetic rats. However, the variation in milling time between 5 and 10 minutes did not significantly influence the extract's effectiveness. Further research with longer intervention periods, standardized phytochemical content, and inclusion of a positive control is recommended to validate its therapeutic potential.

Keywords: Avocado Seed Extract; Blood Glucose; Diabetes Mellitus; Sprague Dawley Rats; Milling Duration

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder marked by elevated blood glucose levels. Globally, DM prevalence was 8.5% in 2014[1] and is projected to rise to 9.9% by 2045.[2] In Indonesia, the prevalence was 7% in 2016, contributing to 6% of deaths.[3] Since DM is not self-limiting, consistent treatment is necessary to prevent complications. While pharmaceutical antidiabetic drugs are available, many are associated with side effects such as hypoglycemia.[4,5]

Herbal treatments have emerged as alternatives due to their potential to minimize side effects. Avocado seeds, which are often considered waste, have been found to contain various phytochemical compounds with potential health benefits.[6] A study by Alhassan et al. demonstrated that avocado seed extract possesses blood glucose-lowering properties.[7] Similar findings were reported by Oktaria et al. and Jiwintarum et al., who observed antidiabetic effects of avocado seed extract in experimental animals.[8,9] However, these studies did not examine the influence of milling duration on extract effectiveness. The variation in milling duration was conducted with the aim of reducing the particle size of avocado seeds.A longer milling time is expected to result in smaller particle sizes, thereby increasing the surface area exposed to the solvent during extraction. The greater surface area enhances solvent penetration and facilitates the release of bioactive compounds, potentially leading to higher extraction yields of active phytochemicals. Therefore, this study aims to determine whether milling time variation (5 vs. 10 minutes)

affects the ability of avocado seed extract to lower blood glucose levels.

METHODS

The avocado seeds were ground with varying milling durations of 5 and 10 minutes. Assuming constant energy per unit time, increasing milling time increases the total energy input to the material, thereby enlarging the surface area and reducing particle size. Studies on various biological materials, such as horseradish and superfine grinding of fibrous food materials (Zhao et al., 2010; Huang et al., 2012)[10,11], have shown that increasing ball-milling duration consistently decreases particle size and alters physicochemical properties.

This experimental study involved 16 male Sprague Dawley rats (8–12 weeks old, >140g). Rats with preexisting diabetes or illnesses were excluded. The sample size was based on ANOVA's degree of freedom requirement.[12] This study has obtained ethical clearance approval issued by the Faculty of Medicine, Atma Jaya Catholic University of Indonesia, with approval number 09/06/KEP-FKUAJ/2019.

Rats were divided into two groups:

- **Group A**: Received extract milled for 5 minutes.
- **Group B**: Received extract milled for 10 minutes.

Each group included 4 diabetic and 4 normoglycemic rats. Diabetes was defined as blood glucose >200 mg/dL,[13] while normal glucose ranged from 50–135 mg/dL.

Rats underwent a 7-day acclimatization. Initial blood glucose levels were recorded using a glucometer. Diabetes was induced via intraperitoneal injection of alloxan monohydrate at 160 mg/kg BW (first dose) and 80 mg/kg BW (booster dose).[12] The quality of alloxan must be maintained by storing it at a temperature of 2–8°C and protecting it from direct sunlight.[15] Alloxan was dissolved in 0.9% NaCl at 4% concentration and injected immediately after preparation to preserve activity.[16]

Glucose levels were measured 48 hours post-injection to confirm diabetes status.[7] Avocado seed extract (250 mg/kg BW) was administered orally for 7 days.[17] The extract was prepared using maceration and derived from the *Ijo Bundar* avocado variety, known for high phenol content.[18]

Statistical analysis was performed using software. Normality was tested with the Shapiro-Wilk method. For normally distributed data, paired t-tests were applied; for non-normal data, the Wilcoxon matched-pairs signed-rank test was used. Intergroup comparisons were conducted using the Mann-Whitney U test, with a significance level of 95%.

Study Design

This study was conducted using an in vivo experimental research design.

Subject and Sampling method

The sample for this study consisted of 16 rats from the species *Rattus norvegicus*, Sprague Dawley strain, which met the following criteria:

Inclusion Criteria

1. Male rats of the *Rattus norvegicus*, Sprague Dawley strain
2. Aged between 8–12 weeks
3. Body weight greater than 140 grams

Exclusion Criteria

1. Rats that had pre-existing diabetes prior to alloxan induction
2. Rats that were visibly ill at the start of the study

Dropout Criteria

1. Rats that became ill during the course of the experiment
2. Rats that died during the experiment
3. Rats that refused to eat during the experiment

Statistical Analysis

Data from this study were first tested for normality using the Shapiro-Wilk test. The significance of blood glucose level reduction was analyzed using the paired *t*-test for normally distributed data, and the Wilcoxon matched-pairs signed ranks test for non-normally distributed data. Comparison between treatment groups was conducted using the Mann–Whitney U test.

studies was systematically documented in a PRISMA flowchart, which is included in this review to enhance transparency and reproducibility.

RESULTS

Both 5-minute and 10-minute milling extracts lowered blood glucose levels in diabetic rats. The mean glucose reduction was:

Table 1. Mean Blood Glucose Levels (Small Test Group)

Milling duration		Blood Glucose Level (mg/dL)			Δ Blood Glucose Level (after *alloxan* injection and after seed extract administration)
		After adaptation	After Alloxan Injection	After Seed Extract Administration	
5 menit	Diabetes	117	403,75	399,75	4
	Normal	114,75	108	61,75	46,25
10 menit	Diabetes	105,75	370,5	272,75	97,75
	Normal	122	102,5	108,5	-6

Table 2 Mean Blood Glucose Level

Milling Duration	Post-Alloxan Glucose (mg/dL)	Post-Extract Glucose (mg/dL)	Δ Glucose (mg/dL)
5 minutes	255.88	230.75	25.13
10 minutes	236.50	190.63	45.88

The reduction in blood glucose levels observed in rats treated with avocado seed extract milled for 5 minutes was not statistically significant (p = 0.2334). Similarly, the group that received extract milled for 10 minutes also exhibited a non-significant reduction in blood glucose levels (p = 0.7794). Furthermore, the difference in glucose-lowering effect between the two milling durations was not statistically significant (p = 0.5995).

DISCUSSION

A decrease in blood glucose levels was observed in all treatment groups in this study; however, statistical analysis showed that the reduction was not significant. This indicates that although the avocado seed

extract may exert a hypoglycemic effect, the magnitude of change was not sufficient to achieve statistical significance. Notably, when comparing the two treatment groups, the extract prepared with a longer milling duration exhibited a greater mean reduction (Δ) in blood glucose levels compared to the shorter milling group. Despite this apparent difference in effect size, the result remained statistically non-significant.

This phenomenon could be attributed to several factors. First, the small sample size may have reduced the statistical power of the analysis, limiting the ability to detect a true effect. Second, variability within the groups—such as individual biological differences in response to both alloxan and the extract—may have contributed to high standard deviations, thus diluting the apparent differences between groups. Third, although a longer milling time may increase the release of active compounds such as phenols, the increase might not have been substantial enough to cross the threshold of statistical significance within the study's timeframe.

In contrast to the present findings, A. A. Aibiremolen et al. reported a statistically significant reduction in blood glucose after 28 days of extract administration.[19] The much shorter administration period in this study only seven days may have been inadequate for the bioactive compounds to exert their full therapeutic effect.

Moreover, all rats in this study, both diabetic and normoglycemic, received two intraperitoneal injections of alloxan.[20] While this method was intended to induce diabetes, it was observed that some rats remained normoglycemic. This outcome reinforces the well-documented inter-individual variability in alloxan sensitivity,[21] which can lead to inconsistent diabetic induction and contribute to variability in blood glucose measurements.

The potential hypoglycemic activity of avocado seed extract may be linked to its phenolic content.[22] The presence of phenolic compounds was confirmed through a ferric chloride ($FeCl_3$) test, which yielded a positive reaction indicated by a greenish-black color change. While phenolic compounds are known to exhibit antidiabetic effects through mechanisms such as antioxidant activity and modulation of glucose metabolism, it is possible that the extract concentration or bioavailability achieved within the short experimental

window was insufficient to produce a statistically significant effect.

CONCLUSION

Avocado seed extract with milling durations of 5 and 10 minutes exhibited equivalent effectiveness in lowering blood glucose levels in experimental rats.

Conflict of interest

Competing interests: No relevant disclosures.

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