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Exploring the Unpredictable: Aloe vera L.Burm.f Extract's Non-Monotonic Dose-Response in Regulating Blood Cholesterol in a Diabetes Mellitus Rat Model

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ABSTRACT: Diabetes mellitus (DM) is a metabolic disorder often accompanied by complications such as elevated blood cholesterol levels. Aloe vera L.Burm.f has long been recognized for its anti-inflammatory and antioxidant activities, but its potential as a therapeutic agent for lowering blood cholesterol in diabetes mellitus rat models has not been fully understood. This study aimed to investigate the effects of Aloe vera extract on blood cholesterol reduction using a diabetes mellitus rat model. Diabetes was induced in rats using Alloxan, followed by oral administration of Aloe vera extract doses 250, 300, and 350 mg/kg BW for 21 days. Blood cholesterol levels were measured by the clinical photometer. The research findings indicate that the administration of Aloe vera extract resulted in a non-monotonic dose response in reducing blood cholesterol levels in diabetes mellitus rats ($P<0.05$). Although the 250 mg/kg BW dose showed a significant decrease approaching normal values, the 300 mg/kg BW dose led to a significant increase, while the 350 mg/kg BW dose again showed a significant decrease. These findings highlight the complexity of the interaction between Aloe vera extract and cholesterol regulation in diabetes mellitus. Therefore, a deeper understanding of these mechanisms is crucial to support the development of more effective and safe therapies in humans.

KEYWORDS: Aloe vera, Alloxan, Blood Cholesterol, Diabetes Mellitus, Non-Monotonic Dose-Response.

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from abnormalities in insulin secretion, insulin resistance, or both, along with progressive changes in the pancreatic beta-cell structure. DM is a chronic disease identified by blood glucose levels exceeding normal values, with fasting blood glucose levels, equal to or greater than 126 mg/dL, and random blood glucose levels equal to or exceeding 200 mg/dL [1].Recognized as a silent killer, DM often goes unnoticed, and complications are discovered only after they have occurred [2].

Long-term complications of diabetes include retinopathy, nephropathy, neuropathy, and other associated complications. Individuals with diabetes are also at a higher risk of developing cardiovascular diseases, peripheral artery disease, cerebrovascular diseases, obesity, cataracts, erectile dysfunction, and liver diseases. WHO reports indicate that only 50% of DM patients in developed countries adhere to prescribed treatments. Uncontrolled DM can lead to severe complications, affecting quality of life and economic productivity [3].

Dislipidemia is a metabolic disorder characterized by abnormal lipid levels in plasma, including elevated total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and reduced high-density lipoprotein (HDL) cholesterol. The linear relationship between increased levels of LDL cholesterol and cardiovascular damage is evident in diabetic patients, as observed in statin intervention trials [4]. However, interventions using fibrate therapy to target triglycerides and HDL cholesterol levels have shown inconsistencies in HDL intervention trials [5].

The inclination of the community to use alternative treatments for various diseases and health disorders is increasing. A global trend toward the use of complementary and alternative medicine, particularly biologically based therapies (nutritional supplements, herbal products, and plant-based treatments) has been observed [6]. One such medicinal plant with anti-diabetic effects is Aloe vera L.Burm.f. (Figure 1), widely used for its therapeutic properties. Aloe vera is a functional

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plant, and all parts of the plant can be utilized for body care, food processing, and the treatment of various diseases, including lowering blood sugar levels in diabetes patients.

Figure 1. Aloe vera L.Burm.f.

Assuming that the administration of Aloe vera can protect and restore damaged pancreatic beta cell function, the components of Aloe vera may act similarly to insulin, reducing blood glucose levels even when all pancreatic beta cells have undergone degeneration[7]. The concentration of Aloe vera gel in the fruit peel depends on the leaf part, age, leaf position on the plant, leaf orientation, and harvesting season. The gel consists of approximately 99.5% water, with the remaining 0.5–1% being solid substances [8].

Based on Wanaditri et al.'s study, the administration of Aloe vera ethanol extract at doses of 250, 300, and 350 mg/kg BW influenced the reduction of blood glucose levels in streptozocin-induced hyperglycemia rat models [9]. According to Rajasekaran et al., a significant reduction in fasting blood glucose levels in rats was observed with the administration of Aloe vera extract[10]. Based on the information provided, this study aims to determine the effect of Aloe vera extract administered on total cholesterol in rats with diabetes mellitus induced by alloxan.

II. METHODS

2.1 Sample Collection

The sample used was Aloe vera weighing 2 kg, obtained from Aie Pacah, Koto Tangah, Padang City, West Sumatra, Indonesia.

2.2 Plant Identification

Plant identification was conducted at the Herbarium of Universitas Andalas (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA), Universitas Andalas, Padang.

2.3 Sample Preparation Process

The sample preparation process involved the following steps: sample collection, wet sorting, washing, slicing, and drying.

2.4 Extract Preparation

The Aloe vera extract was prepared through maceration using a 96% ethanol solvent. One kilogram of the sample was placed in a dark bottle, and 10 liters of the solvent were added for soaking for the initial 6 hours with occasional stirring. Subsequently, it was left to stand for 18 hours at room temperature. The mixture was then separated by filtration using a flannel cloth. The maceration process was repeated at least twice with the same type and amount of solvent. Following that, it was concentrated using a rotary evaporator until a thick extract was obtained. The yield was calculated as the percentage of the extract weight relative to the weight of the plant material used for weighing.

2.5 Thin-Layer Chromatography Analysis

Approximately 10 mg of Aloe vera extract was accurately weighed and soaked while shaken with 1 ml of ethyl acetate for 10 minutes. It was eluted with ethyl acetate: methanol: water (9:1:0.5) as the mobile phase, and silica gel 60 F_{254} as the stationary phase. The spots were observed under visible light, short-wavelength ultraviolet (254 nm), and long-wavelength ultraviolet (366 nm). The distance of each spot was measured and recorded from the spotting point, and the wavelength for each observed spot was recorded to determine the Rf value.

2.6 Phytochemical Screening

a) Alkaloid Test:

A total of 500 mg of the extract was weighed, and 1 mL of 2 N hydrochloric acid and 9 mL of water were added. It was heated in a water bath for 2 minutes, cooled, and filtered. Three drops of the filtrate were transferred to a test tube, and 2 drops of Bouchardat's LP reagent were added. If no precipitate formed in both trials, the extract did not contain alkaloids. If a brown-to-black precipitate formed with Bouchardat's LP, alkaloids were likely present.

b) Flavonoid Test:

Evaporated 1 ml of the extract to dryness, and the residue was dissolved in 1 ml to 2 ml of ethanol (95%).

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c) Steroid and Terpenoid Test:

0.1 gram of the extract was dissolved in chloroformwater (1:1), shaken, and allowed to stand until two layers formed. The resulting solution was filtered, and the filtrate was dripped into a test tube, followed by the addition of Bouchardat's reagent. The formation of a red/purple color indicated the presence of terpenoids, while a green/blue color indicated the presence of steroids.

d) Phenolic Test:

2 mL of the extract was dissolved in 10 mL distilled water. A portion of the solution was transferred to a test tube and added with 2 drops of ferric chloride solution. The formation of a blue or purple-black color indicated the presence of phenolics.

e) Saponin Test:

2 mL of the extract was placed in a test tube, then 10 mL of distilled water was added. The mixture was vigorously shaken until foam formed. If the foam did not disappear upon adding 1 drop of 2 N hydrochloric acid, saponins were present.

2.7 In Vivo Procedure

The experimental animals used were male white rats (Rattus norvegicus) of the Wistar strain, aged 2-3 months, with a weight of 150-200 grams, totaling 20 rats. The rats were acclimatized for 7 days, during which their weights were recorded before and after acclimatization, and they were provided with adequate food and water.

In this study, rats were grouped into 5 treatment groups, with 4 groups induced by alloxan 80 mg/kgBW and one group not induced by alloxan, serving as the normal control. One group served as the negative control and three groups received Aloe veradoses250, 300, and 350 mg/kg BW. The normal control group was given Na CMC throughout the study. The negative control group was induced with alloxan and given a 10% glucose solution for two days after alloxan administration on the second and third days. The extract was administered until day 21 with doses according to each group. Before alloxan induction, the rats fasted for 8 hours and were given a 10% glucose solution the next day, and on day 22, blood glucose was measured using the clinical photometer.

2.8 Measurement of Blood Cholesterol Levels

Serum blood (10 µL) was pipetted into a test tube, followed by the addition of 1000 µL of cholesterol reagent solution . The mixture was allowed to stand for 10 minutes at 37° C. Absorbance was measured at a wavelength of 546 nm against a blank using a photometer (Riele) 5010 v5+. A mixture of cholesterol reagent (100 µL) and distilled water was used as the blank. Standard absorbance measurements were taken, like total cholesterol measurements, with the serum blood replaced by a cholesterol standard.

2.9 Data Analysis

The data wererepresented as the mean ± SD and it wasobtained statistically processed using SPSS Statistic 27.

III. RESULTS AND DISCUSSION

The plant was identified at the Herbarium of Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (MIPA), Andalas University, Padang. The identification confirmed the plant as Aloe veraL.Burm.f belonging to the Xanthorrhoeaceae family.

The extraction process started with the collection of 2 kilograms of Aloe vera leaves. Wet sorting was performed to remove impurities from the Aloe vera flesh. Subsequently, washing was done using clean water to separate impurities still attached to the leaves. The flesh was then separated, washed, and sliced. After washing, Aloe vera flesh was sliced.

Extraction of Aloe vera flesh was performed using the maceration method. This method was chosen for its simplicity, not requiring special equipment or heating, which could potentially degrade or evaporate certain compounds. Maceration involved soaking 2 kilograms of Aloe vera flesh in a dark-colored glass bottle, using ethanol 96% as the solvent. The soaking lasted for 24 hours, stirring every 6 hours in the first 6 hours and repeated three times. This process aimed to extract active compounds present in the plant. The collected macerate was then concentrated using a rotary evaporator to evaporate the solvent and remaining water, resulting in a dense extract weighing 41.0693 grams.

The characterized extract exhibited a brown color, bitter taste, no odor, and a thick consistency in the organoleptic evaluation. This evaluation, based on sensory perception, served as an initial subjective assessment.Thin-layer chromatography (TLC) aimed to identify secondary metabolites present in the extract based on the polarity levels of Aloe vera leaf extract and the solvent used. Two green spots were observed on

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the TLC plate, indicating the presence of compounds in Aloe vera extract, with Rf_1 at 0.23 and Rf_2 at 0.46. The different Rf values could be attributed to varying secondary metabolite contents, leading to different spot separations.Phytochemical screening was conducted to provide insights into the groups of secondary metabolites present in the plant. The results in Table I showed that there were alkaloids, flavonoids, phenolics, and saponins were detected in Aloe vera extract through the screening analysis.

The utilization of animals in this research was conducted under ethical guidelines and was approved by the Ethics Committee of the Medicine Faculty, Andalas University (No. 469/UN.16.2/KEP-FK/2021). Twenty rats were acclimatized for seven days. The acclimatization aimed to allow the animals to adapt to their environment, ensuring they remained healthy and exhibited normal behavior. Alloxan induction was performed with a dose of 80 mg/kg BW intraperitoneally.

Table I. Phytochemical Analysis Results of Aloe vera Extract

No	Chemical Content	Observation Results
1	Alkaloid	Positive
2	Flavonoid	Positive
3	Phenolic	Positive
4	Saponin	Positive
5	Terpenoid	Negative
6	Steroid	Negative

Before alloxan induction, blood glucose levels were checked using a Glukocheck device. After alloxan induction and glucose solution administration, blood glucose levels were rechecked. Rats with blood glucose levels ≥ 126 mg/dL continued with Aloe vera extract suspension administration with doses of 250, 300, and 350 mg/kg BW until day 21.

On day 22, blood was collected from the rats through the orbital sinus and centrifuged. The serum was separated for cholesterol level measurement. Total blood cholesterol levels were measured using the clinical photometer. The average total cholesterol values for each group are shown in Figure 2. The homogeneity test using the Shapiro-Wilk test indicated that the data were homogenously distributed, with a P-value> 0.05. Additionally, the normality test showed that the data were normally distributed, with P-value>0.05. Subsequent analysis proceeded to the parametric testone-way ANOVA with Duncan's post hoc test. The ANOVA results revealed a P-value < 0.05, signifying a significant influence on total cholesterol levels due to the administration of Aloe vera ethanol extract.

The administration of alloxan in the negative control indicates an increase in total cholesterol levels. The extract at a dosage of 250 mg/kg BW demonstrates a significant decrease in total cholesterol levels, approaching normal values. However, the 300 mg/kg BW extract dosage leads to a significant increase in total cholesterol compared to the 250 mg/kg BW dose, the normal control group, and the negative control. On the contrary, the 350 mg/kg BW extract dosage shows a significant decrease in total cholesterol compared to levels observed in the normal control group, negative control, and other extract dosages.

The reduction in total cholesterol levels observed in rats treated with Aloe vera extract dose 250 mg/kg BW can be attributed to the presence of secondary metabolites such as flavonoids, alkaloids, phenolics, and saponins. Phenolic acids, such as gallic acid, and flavonoids, such as quercetin contribute to regulating lipid metabolism and adipogenesis [11]. Alkaloids also known can reduce cholesterol by inhibiting the activity of pancreatic lipase enzyme thereby increasing fat secretion through feces [12]. Saponin acts by inhibiting cholesterol absorption to reduce plasma cholesterol levels in experimental animals. These compounds in Aloe vera had potential pharmacology utility to treat hypercholesterolemia.

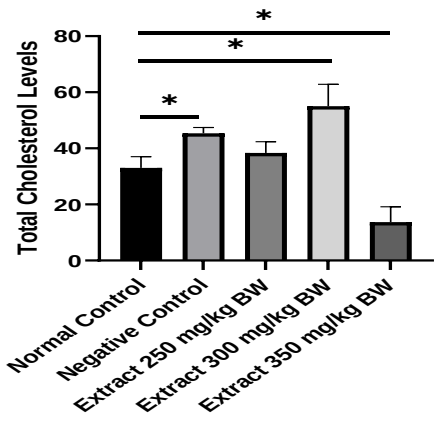

Figure 2. Total cholesterol levels in ras after administration of Aloe vera extract. Each value represents the mean ± SD (n=4).

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The observed fluctuations in total cholesterol levels at the 300 and 350 mg/kg BW extract dosages warrant a closer examination of the underlying mechanisms. The presence of this response is referred to as a non-monotonic dose response. A non-monotonic dose-response refers to a situation in which the effect of a substance or stimulus does not consistently increase or decrease with an increase in dosage.

In this study, it is evident that the effect on total cholesterol levels can vary with the dosage of the plant extract. At a dosage of 250 mg/kg BW, there was a significant decrease in total cholesterol levels. However, at a dosage of 300 mg/kg BW, a significant increase occurred, and at a dosage of 350 mg/kg BW, there was another significant decrease. This pattern indicates a non-monotonic dose response, where the response does not always align with an increase in dosage[13].

The non-monotonicity of the dose-response can be influenced by various factors, including the phytochemical properties, interactions with biological systems, or complex internal regulations.The chemical composition of the plant extract, specifically its phytochemical properties, plays a pivotal role. Different compounds within the extract may have contrasting effects on biological systems at varying concentrations.Certain phytochemicals might exhibit biphasic or multifaceted behaviors, leading to non-monotonic dose responses. These properties can be influenced by the extract's composition, including the presence of secondary metabolites [14].

The way the plant extract interacts with biological systems is also a key determinant. Receptors on cells may respond differently at various dosage levels, leading to divergent effects.Enzyme activities and cellular signaling pathways can be modulated by the extract, and these interactions may exhibit non-linear patterns. The intricate nature of these interactions can contribute to the observed non-monotonic responses.

Internal regulatory mechanisms within the body, aimed at maintaining balance or homeostasis, can influence dose responses. These mechanisms may activate feedback loops or adaptive changes in response to different dosages of the plant extract.Genetic expression and cellular responses to the extract may involve complex regulatory networks. The interplay of these internal regulations can result in non-monotonic patterns.

Given these considerations, further analysis and more in-depth research are imperative to unveil the mechanisms behind the observed non-monotonic dose response to total cholesterol levels. This involves exploring how the plant extract's phytochemical composition interacts with receptors, enzymes, and signaling pathways within the biological systems. Additionally, investigating how internal regulatory processes respond to different dosages is essential for a comprehensive understanding.

By delving into the specifics of these interactions, researchers can gain better insights into the nuanced nature of the relationship between the plant extract and total cholesterol levels. This knowledge not only enhances our understanding of the study's outcomes but also provides a foundation for developing targeted interventions or treatments related to cholesterol regulation.

IV. CONCLUSION

This study sheds light on the potential of Aloe vera L.Burm.f extract as a therapeutic intervention for reducing blood cholesterol levels in a diabetes mellitus rat model. The study reveals a noteworthy non-monotonic dose response, where a lower dosage (250 mg/kg BW) significantly lowers cholesterol levels, approaching normal values. However, at a moderate dosage (300 mg/kg BW), a significant increase is observed, while a higher dosage (350 mg/kg BW) results in another significant reduction.

The observed non-monotonic dose-response underscores the intricate relationship between Aloe vera extract and cholesterol regulation in the diabetes mellitus context. The significant reduction in cholesterol levels at the lower dosage suggests a potential therapeutic benefit. However, the unexpected increase at the moderate dosage and subsequent decrease at the higher dosage highlights the complexity of the dose-response dynamics.

These findings contribute valuable insights into the cholesterol-lowering effects of Aloe vera extract, specifically at the 250 mg/kg BW dosage. Further research is warranted to elucidate the underlying mechanisms driving the non-monotonic response and to assess the long-term safety and efficacy of Aloe vera extract as a potential therapeutic option for individuals with diabetes mellitus susceptible to elevated blood cholesterol levels. The conclusion emphasizes the need for continued investigation to harness the full

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therapeutic potential of Aloe vera in managing cholesterol in the context of diabetes mellitus.

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