



## The Use of Methanolic Extract of *Annona Muricata* Linn Leaves as an Effective Anti-Hyperglycemia on Mice

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### Abstract

Background: Extracts from various morphological parts of soursop leaf (*Annona muricata* Linn/*Annonaceae*) are widely used in many parts of the world for the management, control, and treatment of a human disease, including diabetes mellitus (DM). This study investigates possible anti-hyperglycemia effects of methanolic extract of *Annona muricata* Linn in mice experimental paradigms of DM. Materials and Methods: The mice used were divided into three experimental groups. Group A served as 'control' animals and received sodium citrate buffer solution in quantities equivalent to the administered volumes of methanolic extract of *Annona muricata* Linn. Diabetes mellitus was induced in Groups B and C by intraperitoneal injections of streptozotocin (STZ, 80 mg/kg body weight). Group C were additionally treated with methanolic extract of *Annona muricata* Linn (100 mg/kg body weight, PO) for two weeks. Results: A statistically significant lower blood glucose level was found in Group C compared to Group B in sixth ( $215.14 \pm 78.37$  vs  $319.57 \pm 135.92$  mg/dL) and seventh week ( $158.57 \pm 24.23$  vs  $238.85 \pm 51.35$  mg/dL) of observation. Conclusion: Daily dose of 100 mg/kg body weight methanolic extract of *Annona muricata* Linn administration lowers blood glucose compared to those who did not receive in mice.

**Keywords:** *Diabetes mellitus, Blood glucose level, Soursop leaf, Experimental.*

### Introduction

Type 2 diabetes mellitus (T2DM) is one disease with increasing prevalence all over the world. One factor that affects hyperglycemia in T2DM is insufficiency or failure of beta pancreas cells caused by increased of free radicals. The treatments of T2DM with anti diabetic agents are relatively expensive and may cause side effects. Therefore a breakthrough in alternative medicines that may reduce hyperglycemia is needed. Currently, herbs have been studied as alternative medicines, including soursop leaf (*Annona muricata* Linn)[1]. As a traditional medicine, soursop leaves are easy to find and are often used for cancer drugs.[2] It contains saponin, polyphenol, and bioflavanoid that act as antioxidant to suppress the growth of abnormally developed body cells, such as cancer cells [3].

These antioxidants content should also play a beneficial role in degenerative diseases such as T2DM. This study aims to verify methanolic extract of *Annona muricata* Linn decreased blood glucose level in mice.

### Materials and Methods

This study has been reviewed and was approved by the Committee of Ethical Research of our University. All soursop leaves were collected from a farm in Dalung village at Badung Regency of Bali. Processing of methanolic extract was conducted with Christy and Norris method.<sup>1</sup> Twenty one mice with body weight ranging from 200-250 g were included in the study. They were each assigned to one of three groups so that each group has 7 mice. Group A is control health mice with no diabetes. Group B is mice which induced diabetes without intervention.

Group C is mice which induced diabetes with intervention (treated using extract). Mice in group A were injected with sodium citrate buffer as control. Both groups B and C were induced to diabetes condition by injecting 80 mg/kg body weight single dose

streptozotocine (STZ) intraperitoneally. After two weeks, mice in group C received daily extract 100 mg/kg body weight orally for two weeks. Blood glucose levels were then measured weekly for the next five weeks.

**Table 1: Study group classification**

Group	Label	Hyperglycemia induced	Treatment
A	Control group	None. (Sodium citrate buffer solution was used as control)	N/A
B	No treatment group	Intraperitoneal injection of 80 mg/kg body weight single dose STZ	None
C	Treatment group	Intraperitoneal injection of 80 mg/kg body weight single dose STZ	Daily 100 mg/kg body weight extract orally for two weeks

Mice weights were measured using special scale for mice. For fasting blood glucose level, all mice were put in 16 hours fasting period. Blood samples were collected from orbital vein, and blood glucose measured by glucometer in mg/dL unit. Collected data was analysed descriptively and inferentially. All values were reported in mean  $\pm$  SD in each group. The data was put into anova test, in order to see if the results were affected from our intervention.

## Results

Table 2 shows the mean blood glucose level for each group in each week. Shapiro-Wilk test is used for normality test, and all data were normally distributed ( $p > 0.05$ ). Levene's test is then used to assess the homogeneity of variance. All data variance were homogenous ( $p > 0.05$ ).

**Table 2: Weekly mean plasma blood glucose<sup>1</sup> (p-value<sup>2</sup>)**

Group	Pre treatment	Week 3	Week 4	Week 5	Week 6	Week 7
A	129.85	131.14 (0.923)	133.85 (0.246)	132.00 (0.743)	132.00 (0.981)	123.14 (0.277)
B		459.85 (0.122)	372.14 (0.126)	358.28 (0.497)	319.57 (0.288)	238.85 (0.649)
C		419.57 (0.095)	351.57 (0.228)	309 (0.232)	215.14 (0.230)	158.57 (0.303)

<sup>1</sup>presented in mg/dL <sup>2</sup>Shapiro-Wilk test

Table 3 shows that the mean blood glucose level in group C was lower compared to the

other two groups. The difference is significant in week 6 and week 7 ( $p < 0.05$ ).

**Table 3: Weekly mean plasma blood glucose between diabetes-induced groups (mean $\pm$ SD)**

	Week 3	Week 4	Week 5	Week 6	Week 7
Group B	459.86 $\pm$ 146.17	372.14 $\pm$ 150.74	358.28 $\pm$ 142.82	319.57 $\pm$ 135.92	238.85 $\pm$ 51.35
Group C	419.57 $\pm$ 174.45	351.57 $\pm$ 139.26	309.00 $\pm$ 137.33	215.14 $\pm$ 78.37	158.57 $\pm$ 24.23
p-value	0.57	0.74	0.43	0.04*	<0.001*

\* $p < 0.05$

## Discussion

Diabetes is a chronic metabolic disruption due to various causes marked with hyperglycemia and insulin insufficiency. This insulin insufficiency happens because impaired production by beta cells of the pancreas, or altered responsiveness from the body itself to insulin [4]. Insulin secretion impairment may happen due to beta cells damage involving autoimmune reaction by  $T_h$  cells causing increased blood glucose level [5]. Streptozotocine (STZ) is a nitrosureic

agent that is specifically cytotoxic to beta pancreas cells. It increases the expression of inflammatory cells (TNF- $\alpha$ , IFN- $\gamma$ , IL-18) resulting in hyperglycemia [6, 7]. The implication of STZ administration to mice can be seen in both B and C groups, showing an increase in blood glucose level. Their mean blood glucose level was 459.85 mg/dL and 419.57 mg/dL by the third week after the administration of single dose STZ, compared to 131.14 mg/dL in group A. Administration methanolic extract of *Annona muricata* Linn in group C result in decreasing blood glucose

level compared to hyperglycemic mice in group B. These results are similar to a study by Adeyemi et al, who reported that methanolic extract of *Annona muricata* Linn has an anti-hyperglycemia effect and also may decrease body weight [1]. This indicates that bioactive substances in methanolic extract of *Annona muricata* Linn may play a role with various mechanisms like increasing insulin secretion and improve proliferations of beta cells. Decreasing of blood glucose level is caused by antioxidant substances in methanolic extract of *Annona muricata* Linn like flavonoid, alkaloid, and polyphenol. These substances possess the ability to bind to free radicals, therefore resulting in regenerating damaged beta cells and increasing functions of beta cells.

They keep the beta cells functioning close to normal, allowing regeneration process to beta cells by mitotic or proliferation and differentiation of ductal and ductular cells[7].Flavonoid lowers blood glucose level by inhibiting phosphodiesterase enzyme so that cAMP level in beta cells increase causing the closure of potassium channel in plasma membrane [8].This causing membrane depolarization and in turn opening calcium channel, so that calcium ions enter the beta cells and trigger insulin secretion. These events will cause blood glucose level will slowly decreasing [9].

Other study mentioned that quercetin, a flavonoid substance from flavonol subclass found in soursop leaves, possess a hypoglycemic property by inhibiting alpha amylase enzyme in carbohydrate breakdown [10].In vitro study showed that quercetin also potentially inhibit glucose transport by GLUT2 and GLUT5, which are responsible to glucose absorption in intestine. These

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mechanisms are thought to be the reasons why quercetin may decrease blood sugar level in mice [10].Alkaloid is an antioxidant that possess regeneration ability, where alkaloid extract is capable in regenerating damaged beta cells. An increase in insulin secretion is caused by simpatomimetic stimulation from alkaloid. It is also thought that alkaloid inhibits glucose absorption in the intestine, although the mechanism is still unclear [11].Tannin is a polyphenol that lower blood glucose level by increasing glucose uptake from the activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) [12]. Hydrolyzed tannin is differentiated into gallotannin and ellagitannin.

Gallotannin alleviates glucose uptake and also inhibits adipogenesis. Ellagitannin derivatives (lagerstroemin, flosin B, and regin A) possess insulin-like compound, where in vitro they may alleviate glucose transport mechanism into adipose cells [13]. More studies are needed in order to explain more about these bioactive components in methanolic extract of *Annona muricata* Linn, specifically which of these substances play the most significant role in lowering the blood glucose level. An answer to that question may become an alternative to the management of diabetes mellitus.

## Conclusion

The daily 100 mg/kg body weight methanolic extract of *Annona muricata* Linn administration for two weeks lowers blood glucose level compared to those who did not receive in mice.

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