

THE POTENTIAL OF SHRIMP SHELL EXTRACT AND SOURSOP LEAVES AS AN ANTIDIABETES AGENT IN MICE

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ABSTRACT

Blood glucose levels that remain high over a prolonged period can increase the risk of diseases such as diabetes. Astaxanthin and chitosan from whiteleg shrimp shells and flavonoids from soursop leaves have the potential to lower blood sugar by enhancing insulin sensitivity and inhibiting glucose absorption. This study aimed to assess the potential of shrimp shell extract (SSE) and soursop leaf extract (SLE) as antidiabetic agents in mice. A completely randomized design (CRD) experiment was conducted using 24 male mice (body weight 20-30 g, aged 3-4 months). Twenty mice were induced with hyperglycemia using alloxan at 150 mg/kg body weight, while four mice served as the control. Mice were divided into 6 groups (4 mice each): K1 (control), K2 (Alloxan induction), K3 (SSE 37.5 mg/kg BW), K4 (SSE 42.5 mg/kg BW), K5 (SLE 350 mg/kg BW), K6 (SLE 450 mg/kg BW). Treatments were administered for 14 days, and blood glucose levels were measured on Day 0 and Day 14 using a glucometer strip test. Results showed the highest blood glucose reduction in group K4 (83.81%). Normality and homogeneity tests indicated the data were normal and homogeneous ($P > 0.05$). One-way ANOVA analysis ($F_{hit} \alpha = 0.01 > F_{tab}$, $9.53 > 4.25$, $P < 0.01$) revealed that SSE and SLE had a very significant effect as antidiabetic agents in lowering mice blood glucose levels. Duncan's post-hoc test confirmed K4 and K6 as the most significant groups. It can be concluded that SSE (42.5 mg/kg BW) and SLE (450 mg/kg BW) are effective in lowering blood glucose levels in mice and hold potential to be developed as antidiabetic agents for managing diabetes mellitus.

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Introduction

The liver and skeletal muscles store blood glucose derived from carbohydrate breakdown in the form of glycogen (Samsuri et al., 2020). Carbohydrates, including monosaccharides, disaccharides, and polysaccharides, are converted into glucose in the liver to supply the body with energy (Rosares & Boy, 2022). Hyperglycemia, defined as blood glucose levels exceeding 200 mg/dL two hours postprandially, is often an early indicator of diabetes mellitus, a chronic metabolic disorder impairing glucose regulation (Putri & Situngkir, 2022). If uncontrolled, diabetes can cause cardiovascular, ocular, renal, neural, and cardiac complications. Type 2 diabetes, which is common in adults, occurs as a result of insulin resistance or deficiency (Prastiwi et al., 2024). Diabetes Mellitus causes about 1.5 million deaths a year and affects 422 million people, especially in low- and middle-income countries. (WHO, 2024). The International Diabetes Federation (IDF) reports that the global prevalence of

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diabetes continues to increase and is estimated to reach 700 million people by 2045, including in developing countries such as Indonesia.

Marine resources abound in Indonesia, which is a maritime country, including shrimp, specifically Vannamei shrimp or White-footed Shrimp (*Litopenaeus vannamei*), which are rich in protein, minerals, and chitin. These shrimp shells contain chitin (chitosan), crude proteins, and minerals, as well as active compounds such as astaxanthin and enzymes (Huang et al., 2022; Widyastuti, 2023). Astaxanthin is known to help control blood sugar levels by optimizing insulin sensitivity and release, as well as reducing diabetic complications such as retinopathy, nephropathy, and neuropathy (Prasetyaningsih et al., 2023). In addition, shrimp shell contains chitosan, a natural polymer that plays a role in protecting and multiplying pancreatic beta cells, lowering hyperglycemia, and preventing fat metabolism disorders (Sarkar et al., 2020) Chitosan inhibits alpha-glucosidase in the intestine, namely by decreasing the activity of enzymes (maltase, sucrase, and lactase) thereby slowing down the breakdown of carbohydrates and reducing post-meal hyperglycemia (Dahlén et al., 2022; You et al., 2022). A study (Wisaksono et al., 2021) that tested blood glucose reduction with shrimp shell extract (*Litopenaeus vannamei*) at a dose of 37.5 mg/kg BW showed the best results, The average blood glucose level can be reduced from 429.8 mg/dL to 140.6 mg/dL.

In addition to chitosan and astaxanthin from shrimp peel extract which are able to reduce glucose levels in the blood, plants and natural ingredients can be used as potential alternatives as antidiabetic drugs. Soursop leaves (*Annona muricata* L.) are among the plants that exhibit antidiabetic behavior (Agu et al., 2019). Soursop leaves have an antihyperglycemic effect because they contain flavonoids, alkaloids, saponins, and tannins (Purnamasari et al., 2021). Blood glucose levels are reduced with flavonoids, which increase glucose tolerance and inhibit the flow of glucose through intestinal glucose transporters, and stimulate cells β pancreas to secrete insulin production (Fadel & Besan, 2020 production & Issusilaningtyas et al., 2024). Tannins act as free radical antibodies, help reduce oxidative stress, control blood sugar and prevent complications among diabetic patients (Hasan et al., 2024; Riza et al., 2023). Similarly, for soursop leaves, (Sindi et al., 2022) stated that soursop leaf extract therapy for 14 days in aloxan-induced mice was able to reduce blood glucose levels, with the most significant effect at a dose of 400 mg/kg BW, but it had not been able to lower it to the normal range of blood sugar levels (70-99 mg/dL).

Experimental research is needed to prove the role of natural ingredients in alternative antidiabetic treatment. This study was conducted to measure the potential of treating shrimp peel extract and soursop leaf extract as antidiabetic agents in mice given alloxan.

Method

Quantitative experimental research was used as a comparison in the results of grouping test animals. A completely randomised design (CRD) with six treatment groups, with four mice each, was conducted at the Biology Laboratory, Integrated Laboratory Unit of Sebelas Maret University, Surakarta. A total of 24 male mice (*Mus musculus* L.) aged 3-4 months with an average weight of 20-30g were used as experimental animals. Twenty mice were induced by alloxan as much as 150 mg/kg BW, and four mice as control. The treatments in this study were; K1 (control), K2 (Alloxan Induction), K3 (SSE 37.5 mg/kgBW), K4 (SSE 42.5 mg/kgBW), K5 (SLE 350 mg/kgBW), K6 (SLE 450 mg/kgBW). In this study, animals were fed with standard mice feed (BR Comfeed) and water that was always available (ad libitum).

The process of making shrimp shell extract (SSE) begins with drying shrimp shells in a weight of 500 g under the sun for \pm 3 days as stock, then smoothing into powder using a blender. Weighing 100 g of fine shrimp shell and macerating the shrimp shell powder using 70% ethanol

for 72 hours as much as 1000 ml (1:10), then filtering the macerate. Evaporate the precipitate obtained using a rotary evaporator at 70°C and then heat the thick shrimp shell extract at 100°C in a waterbath, then weigh the thick paste to calculate the yield. the final stage is to store the resulting thick shrimp shell extract for treatment.

The process of making soursop leaf extract (SLE) begins with drying 500 g of soursop leaves under the sun for ± 3 days as stock, then smoothing into powder using a blender. Weighing the fine soursop leaves as much as 100 g then macerating the soursop leaf powder using 70% ethanol for 72 hours as much as 1000 ml then filtering the macerate. evaporating the fibre obtained using a rotary evaporator at a temperature of 70 ° C then heating the thick soursop leaf sediment with a temperature of 100 ° C in a waterbath weighed to calculate the yield. the final stage is to store the resulting thick soursop leaf extract for treatment.

A total of 24 male mice were acclimatised for one week and given standard mice feed (BR Comfeed) and water that was always available (ad libitum), then the mice were grouped into 6 treatment groups (@= four animals) randomly with the treatment groups listed in (Table 1) for 14 days.

Blood glucose levels were measured practically and quickly by utilising test strips on an Easytouch® glucometer. Blood samples used came from the tail vein of mice. To determine the percentage reduction in blood glucose levels after treatment, it was calculated by the formula:

$$\text{Decrease in sugar content \%} = \frac{\text{KGD Hari ke-0} - \text{KGD Hari ke-14}}{\text{KGD Hari ke-0}} \times 100 \%$$

Table 1. Experimental Design of Shrimp shell Extract and Soursop Leaf Extract Treatment as Antidiabetic Agents on Blood Sugar Levels in Mice (*Mus musculus* L).

No.	Treatment	Alloxan	SSE	SLE
1.	K1	-	-	-
2.	K2	150 mg/Kg BW	-	-
3.	K3	150 mg/Kg BW	32.5 mg/kg BW	-
4.	K4	150 mg/Kg BW	42.5 mg/kg BW	-
5.	K5	150 mg/Kg BW	-	350 mg/kg BW
6.	K6	150 mg/Kg BW	-	450 mg/kg BW

Description : SSE: Shrimp Shell Extract , SLE: Soursop Leaf Extract , K1 (control), K2 (Induction) Alloxan), K3 (SSE 37.5 mg/ kgBW), K4 (SSE 42.5 mg/ kgBW), K5 (SLE 350 mg/ kgBW), K6 (SLE 450 mg/ kgBW)

The measurement data were then tested for normality and homogeneity. Analysis of the average percentage difference in blood sugar levels of each treatment group was carried out by Anova test at 99% confidence level ($\alpha = 0.01$) and Duncan's further test.

Results

The test results of blood sugar levels in mice (*Mus musculus* L.) at the beginning (Day 0) and end (Day 14) of shrimp shell extract (*Litopenaeus vannamei*) and soursop leaf extract (*Annona muricata* L.) (Table 2).

Table 2. Average Results of the Blood Sugar Level Research in Mice (*Mus musculus* L.) After Giving Shrimp Shell Extract And Soursop Leaf Extract For 14 Days.

Treatment Group	Blood Sugar Levels		
	Initial(mg/dL)	End(mg/dL)	Difference (%)
K1	103.75	77.5	26.08^a ± 14,78
K2 ALOKSAN	451	301.25	35.60 ^{ab} ± 9,55
K3 SSE (32,5 mg/kg BW)	455.5	185.75	58.18 ^{bc} ± 13,28
K4 SSE (42,5 mg/kg BW)	498	79.5	83.81^d ± 2,35
K5 SLE (350 mg/kg BW)	316.5	112.5	53.54 ^{bc} ± 20,91
K6 SLE (450 mg/kg bw)	467.5	96.25	78.21 ^{cd} ± 5.96

Description : K1 (control), K2 (Induction Aloxxan), K3 (SSE 37.5 mg/ kgBW), K4 (SSE 42.5 mg/ kgBW), K5 (SLE 350 mg/ kgBW), K6 (SLE 450 mg / kgBW)

Based on (Table. 2) shows the mean blood sugar levels of mice after 14 days of treatment, the highest percentage difference in sugar levels was found in treatment K4 (83.81%) and the lowest in group K1 (26.08%).

The data were then subjected to Kolmogorov-Smirnov and Shapiro-Wilk normality tests at the $\alpha = 5\%$ level. The results of the normality test of mice blood sugar levels through the calculation of SPSS version 23.0 (Table 3) all groups have a significance value of $5\% / \alpha > 0.05$ thus, the data results of blood sugar levels show a normal distribution.

Table 1 Normality Test of Blood Sugar Levels in Mice (*Mus musculus* L.)

Group	Kolmogorov-Smirnov ^a			Shapiro Wilk			
	Statistics	df	Sig.	Statistics	df	Sig.	
Blood Sugar Level	K1	.241	4	.	.965	4	.811
Difference	K2	.188	4	.	.992	4	.967
	K3	.275	4	.	.856	4	.246
	K4	.291	4	.	.786	4	.079
	K5	.202	4	.	.982	4	.914
	K6	.243	4	.	.887	4	.370

a. Lilliefors Significance Correction

The homogeneity test (Table 4), showed that the treatments had a significance value of $\alpha > 5\%$ ($0.208 > 0.05$).

Table 2 Homogeneity Test of Blood Sugar Levels in Mice (*Mus musculus* L.)

		Levene Statistics	df1	df2	Sig.
Blood Sugar Level	Based on Mean	1.611	5	18	.208
Difference	Based on Median	1.546	5	18	.225
	Based on Median and with adjusted df	1.546	5	8.137	.276
	Based on trimmed mean	1.611	5	18	.208

Anova test with f test level (Table 5). The f test at the 0.05 test level obtained the value of $F_{hit} = 9.53$ while the F_{tab} value = 2.77, meaning that $F_{hit} > F_{tab}$ ($9.53 > 2.77$) then 'Ho is rejected', so that 'Ha' is accepted, namely the administration of shrimp shell extract and soursop leaf extract with various doses has an effect as an antidiabetic agent because it can reduce blood sugar levels in mice.

Table 3 ANOVA Test of Blood Sugar Levels in Mice (*Mus musculus L.*)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4035.789	5	807.158	9,533	.000
Within Groups	1524.014	18	84,667		
Total	5559.803	23			

Furthermore, the F0.01 test ($F_{hit} > F_{tab}$) shows the F_{hit} value of 9.53 and the F_{tab} value of 4.25, meaning that $F_{hit} > F_{tab}$ ($9.53 > 4.25$), namely the administration of shrimp shell extract and soursop leaf extract with different dose variations is very influential as an antidiabetic agent because it reduces blood sugar levels in mice (*Mus musculus L.*). Then to see the dose variation group that has the most significant reduction potential, it was analysed using Duncan's further test, with the results presented in (Table 6).

The results of Duncan's further test with an alpha value = 0.01 (Table 6), groups that are in the same subset do not have significant differences in the difference in sugar levels, while groups that are in different subsets have significant differences.

In general Overall, K1 and K2 have a lower difference in sugar levels than other groups, while K6 and K4 have the highest difference in sugar levels. K3 and K5 are in the middle and not significantly different from each other, so the groups that really have an effect on reducing the blood sugar levels of mice are K4 and K6.

Table 6. Duncan's Advanced Test Results Difference Group Shrimp Shell Extract and Soursop Leaf Extract To Decrease in Blood Sugar Levels in Mice (*Mus Musculus L.*)

	Group	N	Subset for alpha = 0.01			
			1	2	3	4
Waller- Duncan a,b	K1	4	29.72812401			
	K2	4	36.44706227	36.44706227		
	K5	4		47.39064390	47.39064390	
	K3	4		49.87591480	49.87591480	
	K6	4			62.33536858	62.33536858
	K4	4				66.30291363

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4,000.

b. Type I/Type II Error Seriousness Ratio = 100.

Based on the graph (Figure 1) shows the average blood sugar levels of mice decreased significantly, seen from the difference that is getting bigger. With groups that are very influential in reducing blood sugar levels of mice are K4 and K6.

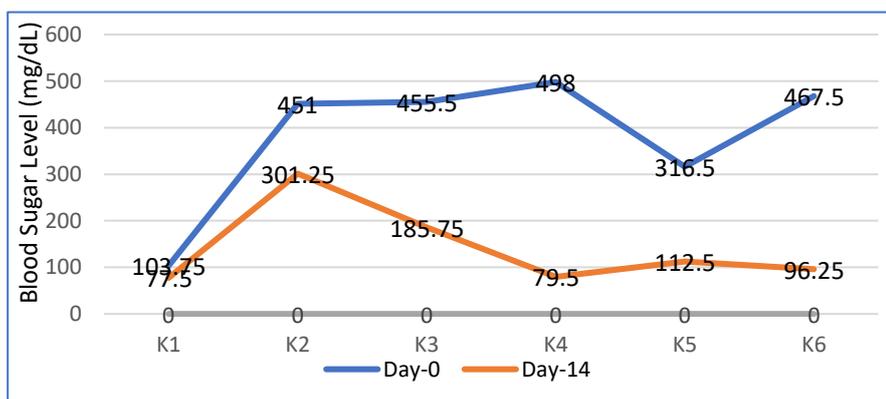


Figure 1. Graph Average Results of Research on Blood Sugar Levels in Mice (*Mus musculus L.*) After Giving Shrimp Shell Extract and Soursop Leaf Extract For 14 Days.

Discussion

Based on the results of Duncan's further test, it is shown that K4 (SSE = 42.5 mg/BW) is the treatment that has the highest percentage difference in blood sugar levels and is not significantly different from K6 (SSE = 450 mg/BW). This shows that the higher the dose of treatment of shrimp shell extract and soursop leaf extract, the higher the difference in blood sugar levels that can be reduced, this research is in line with research (Wisaksono et al., 2021) which reports that the decrease in blood sugar with a dose of shrimp shell extract of 37.5 mg / kg BW shows that blood sugar levels are reduced from 429.8 mg / dL to 140.6 mg / dL (289.2 mg / dL) which means that it can reduce blood sugar levels but not yet close to the normal limit of Fasting Blood Sugar levels (GDP = 70-99 mg / dL). The average blood sugar levels of K4 (SSE = 42.5 mg/BW) from 498 mg/dL to 79.5 mg/dL and K6 (SLE = 450 mg/BW) from 467.5 mg/dL to 96.25 mg/dL both treatment groups have optimal sugar levels.

Shrimp shells contain chitin substances that produce natural polymers, namely chitosan, which has the effect of protecting and multiplying pancreatic beta cells (cells that produce insulin), reducing hyperglycemia (high blood sugar levels), and preventing metabolic disorders (Sarkar et al., 2020). In research You et al (2022) stated that the multitarget modulation ability of COS (Chitosan oligosaccharide), including inhibiting intestinal glucose absorption, accelerating the absorption and use of glucose in the liver and muscles, and improving pancreatic function, which simultaneously regulates glucose metabolism in the body.

Astaxanthin's potential to lower blood sugar levels is in line with research that astaxanthin effectively controls hypoglycemia in animal models of type 1 diabetes mellitus. Astaxanthin is an alternative to antidiabetic natural oral agents (Sztretye et al., 2019; Medoro et al., 2024). In line with Afifah et al (2024) states that astaxanthin is the main carotenoid pigment found in aquatic animals. Astaxanthin from shrimp waste produces significant hypoglycemic effects after oral administration in hyperglycemic rats given alloxan, so astaxanthin can be a useful natural oral agent for treating diabetes

The benefits of soursop leaves are also supported by Opara et al (2021) that soursop leaves have antioxidative effects and have several antioxidant properties that can be important in the treatment and management of disease conditions such as diabetes such as diabetes mellitus, cancer, arthritis, and other disease conditions due to oxidative stress. Soursop leaf extract can reduce blood sugar levels because it is rich in alkaloid compounds, steroids, tannins, and phenolic and flavonoid compounds so that it can also be used as an antioxidant (Asfahani & Anna, 2022).

Soursop leaves contain flavonoid metabolites that have a role in helping to reduce blood glucose levels. Flavonoid compounds have various mechanisms in reducing blood sugar levels, including reducing glucose transport, increasing glucose tolerance, encouraging insulin secretion or functioning to give insulin-like effects, increasing the frequency of glucose use in peripheral tissues, and managing the performance of carbohydrate metabolism enzymes (Dewi et al., 2022). In vitro research on soursop leaves showed that the leaf extract has the ability to inhibit the activation of α -amylase and α -glucosidase enzymes by inhibiting the performance of both enzymes, soursop leaf extract can slow down the breakdown and absorption of complex carbohydrates into glucose, which in turn helps delay the increase in blood sugar levels (Djunarko et al., 2022).

Conclusion

The results and analysis of the research on the administration of shrimp shell extract and soursop leaf extract showed that it has the potential to be an anti-diabetic agent that helps reduce blood sugar levels in mice (*Mus musculus* L.) and a very significant decrease was in the treatment group of shrimp shell extract 42.5 g/kg BW followed by a dose of soursop leaf extract 450 mg/kg BW. Shrimp shell extract and soursop leaf extract have the potential to be studied and utilised as glucose-lowering agents in patients with diabetes mellitus.

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