

## STUDI OF *IN VIVO* ANTIDIABETIC ACTIVITY OF NAMNAM LEAVES (*Cynometra cauliflora*) EXTRACT IN SPRAGUE DAWLEY RAT

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### Abstract

Various treatments for Type 2 Diabetes Mellitus (T2DM) aim to alleviate hyperglycemia. Although natural products have been extensively utilized, their mechanism of action and efficacy as antidiabetic agents, particularly for T2DM, has not been extensively assessed *in vivo*. In this research, the leaves of the Namnam (*Cynometra cauliflora*) were extracted and tested for their activity as an antidiabetic agent. Sprague Dawley rats aged 3.5-4 months, weighing 200-250 g, were used as the experimental model, with a total of 30 individuals. The rats were induced with a high-fat diet and 30% sucrose until their blood glucose concentration reached  $\geq 120$  mg/dL. Subsequently, the rats were divided into four groups (groups 1, 2, 3, and 4). Over 21 days, changes in blood glucose, triacylglycerol, glycogen, and blood plasma insulin levels were assessed. The results demonstrated that the methanol extract of Namnam leaves (NLME) effectively reduced blood glucose levels by 23-34%, decreased plasma triglyceride levels by 13-30%, and increased liver glycogen levels by 68-96% compared to the control group (Diabetes). Among all the parameters assessed, NLME exhibited similar performance to metformin, a commonly prescribed diabetes medication ( $p < 0.05$ ). Furthermore, the study revealed that NLME exerted antidiabetic effects, particularly for T2DM, by promoting liver glycogen formation, enhancing insulin secretion control, facilitating glucose absorption by muscles, and restricting fat metabolism in the blood.

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### INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is indicated by fasting and postprandial hyperglycemic, relative insulin insufficiency [1]. T2DM has frequently been related to morbidity and substantial death [2], 400 million people worldwide are estimated to have this disease in 2030 [3].

Many types of e-treatment are currently used to reduce hyperglycemia in T2DM. For example, sulfonylureas act as insulin secretagogues to increase insulin secretion from the pancreas; metformin to decrease hepatic glucose production; glucosidase inhibitors that interfere with intestinal glucose absorption; and insulin therapy to suppress glucose production and increase glucose utilization by cells. However, the efficacy and tolerability of such therapies are minimal and have significant side effects [4].

For the reasons above, many patients use alternative or complementary therapies to the prescribed medications [5]. These alternative therapies may be in the form of natural medicines considered adequate with minimal side effects in clinical use and relatively low cost. One plant to make this possible is the leaves of Namnam plants (*Cynometra cauliflora*). Traditionally, this plant has been used for various purposes. *C. cauliflora* reduces serum total cholesterol and LDL cholesterol as well as serum lipase and triglycerides [6]. However, the Namnam leaves methanol extract (NLME) capability as an antidiabetic of T2DM still needs further investigation, especially *in vivo* studies.

Therefore, this study focused on the *in vivo* studies of NLME antidiabetic activity test using Sprague Dawley rats. This study series expects reliable data on NLME activities against blood glucose, liver glycogen, triglyceride, and insulin profiles. The results will provide information on

NLME activities as an alternative to T2DM treatment and data on the development of potential plant utilization for treatment in the future.

## EXPERIMENT

### Material

The testing materials used in this study are Namnam leaves (*Cynometra cauliflora*) from Cintaratu village, Parigi, Pangandaran Regency, West Java; rat standard feed and high sucrose and high fat feed; ELISA Rat Insulin Kit (Elabscience); Glucose Kit (Human); Triacylglycerol Kit of liver homogenate (Human); 10% TCA; ethanol absolute 96% (Merck); ethyl ether (Smart Lab); EDTA Merck; and other chemicals. The experimental animals used are 30 male white rats (*Rattus norvegicus*) of Sprague Dawley strain, 3.5–4 months old, 200–250 grams.

### Instrumentation

The apparatus used in this study are glass equipment, blood sugar meter (Easy Touch<sup>®</sup>), micropipette, animal cages (and the accessories), homogenizer Potter Elvehjem, surgical instrumentations, and other supporting equipment.

### Procedure

#### Sample Preparation

Namnam leaves are washed with running water, sorted, and then dried in direct sunlight for 30 hours until the moisture content is 9-10%. Namnam leaf samples (*Cynometra cauliflora*), which had dried, the dried sorted, and then crushed with a blender to obtain a smooth powder. The small sample size (smooth powder) is expected to expand the material surface in direct contact with the solvent to extract bioactive components at the maximum. One hundred grams of Namnam leaf powder (*Cynometra cauliflora*) were soaked in 500 mL of methanol p.a and macerated for 24 hours. After 24 hours, the result of maceration is filtered with Whatman filter paper no.1 so that the filtrate is obtained first. Then the residue leaves macerated Namnam (re-maceration) with methanol p.a were 250 mL for 9 hours to obtain a second filtrate. Furthermore, the first and second filtrates are mixed and concentrated using a vacuum rotary evaporator at a temperature of 45-50°C, so all that remains the leaf extract Namnam forms viscous extract (crude extract).

### Experimental Animals Treatment

Antidiabetic potential testing of Namnam leaves crude extract *in vivo* used healthy male Sprague Dawley rats with active movements, thick white fur, and eyes clear and unblemished. The rats were adapted for 3 weeks by providing standard feed (commercial feed BR512) and aquadest *ad libitum* until the age of 3.5 – 4 months and weighed 200-250 g. The conditions of the experimental animal cages: Temperatures range from 25-28° C and humidity ranges from 70-75%. Light reception is set to 12 hours of light and 12 hours of darkness.

Subsequently, the rats were put according to their body weight and were divided into 4 groups n=3, triplicate). Group 1 were provided with standard feed, while group 2 to 4 were induced with high-fat feed and 30% sucrose (*ad libitum*). The induction was conducted for 11 weeks, during which all groups of treatments 1 to 4 were measured for blood glucose concentration every week using a strep test (Rapid Test Easy Touch<sup>®</sup>). The measurement was performed until the blood glucose level reached  $\geq 120$  mg/dL (insulin resistance) (**Figure 1**). During treatments, high-fat feed and 30% sucrose were still provided every day for 21 days and sampling was conducted every 7 (seven) days. The drug and extract were administered by using a feeding tube. Group 1 was used as the regular group (normal rats with standard feeding), group 2 as negative control (diabetic rats, given 1 mL of distilled water/day, and Group 3 as a positive control of Metformin (diabetic rats treated with metformin) dose of 10 mg/kg body weight (BB)/day, group 4 diabetic rats were given Namnam Leaf Methanol Extract (EMDN) with the selected extract dose of 450 mg/kg BW/day. When the treatment was given, high-fat feed and 30% sucrose were still given every day for 21 days, and sampling was done every 7 (seven) days. Drug administration and treatment by force-feeding method using a sonde.

### Level of Glucose (GOD-PAP Method)

The level of glucose was tested using the glucose oxidase/oxidase method (GOD-PAP). A series of test tubes were prepared for standards, samples, and blanks with compositions as shown in **Table 1**. The ingredients were mixed and incubated for 5 minutes at 37°C. The absorbance of the standard and the sample was measured with a Uv-Vis spectrophotometer at a wavelength of 500 nm against a blank for 60 minutes ( $\Delta$ Absorbance). The glucose concentration was calculated according to the formula:

$$\text{Level of glucose } \left(\frac{\text{mg}}{\text{dL}}\right) = 100 \times \frac{\Delta\text{Absorbance Sample}}{\Delta\text{Absorbance Standart}}$$

**Table 1.** Composition of standard solutions, samples, and blanks of the GOD-PAP method.

Pipette into a test tube	Standart	Sample	Blank Reagent
Standard	10 $\mu\text{L}$	-	-
Sample	-	10 $\mu\text{L}$	-
Blank Reagent	1000 $\mu\text{L}$	1000 $\mu\text{L}$	1000 $\mu\text{L}$

*Level of Triglyceride test (GPO-PAP Method)*

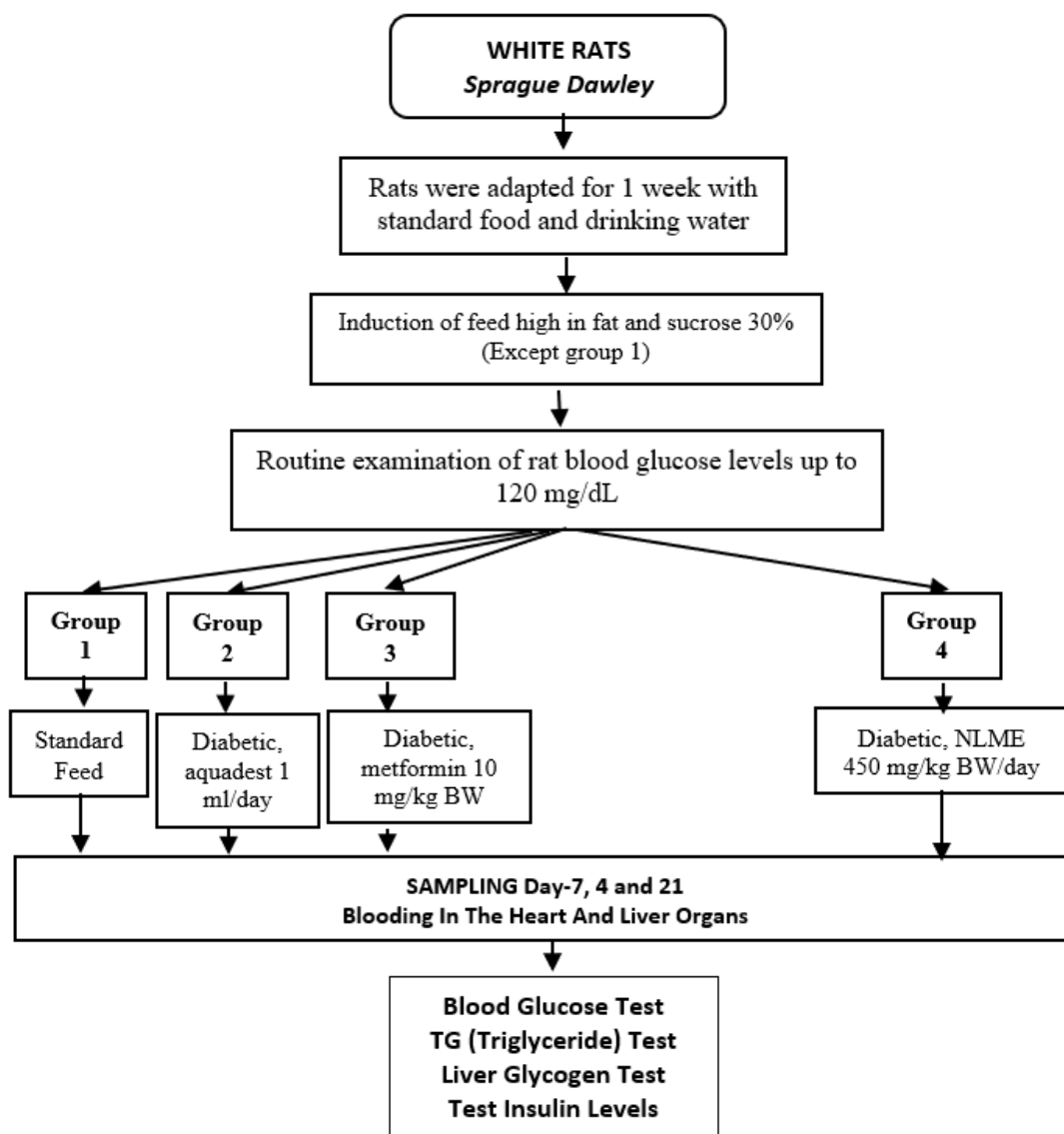
A series of test tubes were prepared for standards, samples, and blanks with compositions as shown in **Table 2**. The ingredients were mixed homogeneously and incubated for 5 minutes at

37°C. The absorbance of the standard and the sample was measured with a Uv-Vis spectrophotometer at a wavelength of 500 nm against a blank for 60 minutes ( $\Delta\text{Absorbance}$ ). The concentration of triglycerides is calculated by the formula:

$$\text{Level of Triglyceride } \left(\frac{\text{mg}}{\text{dL}}\right) = 200 \times \frac{\Delta\text{Absorbance Sample}}{\Delta\text{Absorbance Standart}}$$

**Table 2.** Composition of standard solutions, samples, and blanks of the GPO-PAP method.

Pipette into a test tube	Standart	Sample	Blank Reagent
Standard	10 $\mu\text{L}$	-	-
Sample	-	10 $\mu\text{L}$	-
Blank Reagent	1000 $\mu\text{L}$	1000 $\mu\text{L}$	1000 $\mu\text{L}$



Note: NLME: Namnam Leaves Methanol Extract, BW: Body of Weight

**Figure 1.** The flow and grouping of treatments in this study.

### *Insulin Level Measurement*

The plasma insulin level was measured by using the ELISA (*Enzyme-Linked Immunosorbent Assay*). The measurement was conducted 2 times for every group of samples: Group 1 (normal), Group 2 (Diabetes/Diab), Group 3 (Diabetes + metformin/Diab+Met), and Group 4 (Diabetes + NLME/Diab+NLME). The insulin measured was the insulin in the blood plasma samples of fasting rats. The samples were taken on days 7, 14, and 21.

Insulin measurement used the rat insulin ELISA kit (Elabscience) and the standard reagents was prepared at concentrations of 200, 100, 50, 25; 12.5, 6.25, 3.13, and 0 ng/mL. To the wells, it was added 100 $\mu$ L of sample solutions, standards, and blanks. The blanks were then added with reference and sample solutions. Further, all wells were sealed with the sealers provided and incubated for 90 minutes at 37°C. Solutions were then discarded, and the residue of each well was added with 100  $\mu$ L of Biotinylated Detection Absolution, sealed with the sealers provided again and incubated for 1 hour at 37°C. The wells were washed with about 350  $\mu$ L washing buffer three times. At the end of the washing, the ELISA plate was flipped on absorbent paper to ensure that all washing solutions had been removed. Then, 100 $\mu$ L HRP Conjugate was added to every well, sealed with the sealer, and incubated for 30 minutes at 37°C. The wells were then washed with about 350  $\mu$ L washing buffer 5 times and added with 90 $\mu$ L substrate. The plate was sealed again and incubated for 15 minutes at 37°C. The reaction was stopped with the addition of 50 $\mu$ L stop solution and subsequently read at  $\lambda$  450 nm with an ELISA reader.

### *Glycogen Level Analysis*

The whole rat's liver was weighed and put into a centrifuge tube. Then, 10% TCA (Trichloroacetic Acid) was added (3:2), stirred, and centrifuged at 3000 rpm for 5 minutes. The supernatant was separated and the volume was recorded, and 96% ethanol was then added to the liver extract (1:2) while stirring. If there was no agglomeration, NaCl was added drop-wise and heated in a water bath until precipitation was observed. The mixture was then centrifuged at 3000 rpm for 3 minutes, the supernatant was discarded, and the residue was dissolved in 5 mL aquadest.

The next process was re-precipitation with 10 mL of 96% ethanol by centrifugation, the precipitate was washed with 3 ml of absolute ethanol and ethyl ether and dried on a watch glass. The dried precipitate was then weighted to find out the glycogen formed during the T2DM process compared to the normal control.

### *Data analysis*

Data measurement results were analyzed using Software-SPSS 20.0. The data obtained is treated as related data and proven by a one-way ANOVA test. For the test group that has a significant difference, it is continued with the Tuckey test. The significance limit used was  $p < 0.05$ . The blood sample data obtained were statistically processed using the SPSS software test method to determine significant differences from each group at  $P < 0.05$  to the control group

## **RESULT AND DISCUSSION**

The in vivo antidiabetic activity of Namnam leaves (*Cynometra cauliflora*) extract has been conducted using Sprague Dawley rat as animal models. Rats were made diabetic through high fat and high sucrose (carbohydrate) diet induction. According to Shen and Chang [7], the fasting blood glucose level of rats induced with a high carbohydrate diet increased up to  $129.0 \pm 6.2$  mg/dL in 12 weeks. Wilde et al. [8] showed that a high-fat diet provided for 8 weeks could induce obesity and insulin resistance, increase triglyceride circulation, and rapid hepatic glucose production. Experimental animals provided with a high-fat diet for 8 weeks had already been indicated as chronic to visceral fat accumulation [9].

According to Sylow et al. [10], a high-fat diet will lead to a significant decrease in glucose absorption of skeletal muscles. In addition, Glucose and sucrose supplementation initially resulted in a significant increase in glucose and insulin levels compared to fructose supplementation [11]. Glucose, fructose, and sucrose can all induce lipid deposition in overfed goose liver, however, the regulatory mechanism is different [12].

### *Blood Glucose Analysis*

The data analysis of glucose level in **Table 3** showed that the fasting blood glucose concentration of  $\geq 120$  mg/dL. It indicated that the experimental rats had fulfilled the blood glucose level parameter in the Type 2 Diabetes Mellitus model [13]. The blood glucose range of group 2-4 (a diabetic condition with high fat and 30% sucrose induction) was 121 – 136 mg/dL while the blood range of group 1 (normal, without induction of high fat and 30% sucrose) was 89 – 102 mg/dL. In this study, rats were made diabetic through the induction of a diet high in fat and sucrose

(carbohydrates) which had been carried out by many researchers, either alone or in combination with a mixture of Alloxan or STZ (streptozotocin).

The blood glucose level of diabetic animals (days 7 to 21) treated with metformin (Diab+Met) reduced by about 12-19% and diabetic animals treated with *NLME* (Diab+ *NLME*) reduced by about 23-34%. The Tuckey test did not show any significant difference ( $p < 0.05$ ) between these two treatments. These blood glucose level reductions came to a condition where the levels were insignificantly different ( $p < 0.05$ ) from the normal condition (**Table 3**). This means that the Namnam leaf extract can reduce blood glucose levels just like metformin. Compared to a previous study, the result was almost the same as the results of Fadheel [14] that after three months of treatment, Garlic has made a significant decrease in fasting blood glucose level ( $P < 0.05$ ), as compared to those who used metformin alone. Thus, Garlic has a beneficial

effect on blood glucose levels and can be administered to diabetic patients, in the absence of contraindications.

The result is also in line with the research of Sumarlin et al. [15] which showed that the increase in glucose uptake by diaphragm was discovered when the concentration of Namnam leaf extract (*C. cauliflora*) was increased to 450 mg/mL. In this condition, it can absorb glucose up to  $0.4254 \pm 3.23$  mg/mL/30 minutes. Glucose absorption was found to increase by 373.72% compared to water control. The phytochemical evaluation of *C. cauliflora* leaves showed the presence of flavonoid, saponin, and phenolic compounds [6]. The study of Hajiaghaalipour et al. [16] that the important role of flavonoids in enhancing glucose uptake and expression of glucose transporter proteins in particular up-regulation and translocation of GLUT-4.

**Table 3.** Results of *in vivo* measurement of glucose, and insulin levels.

Group	Glucose (mg/dL)			Insulin (ng/mL)		
	H7	H14	H21	H7	H14	H21
Normal (control)	103.31 ± 5.66 <sup>a</sup>	92.74 ± 3.15 <sup>a</sup>	90.05 ± 8.27 <sup>a,b</sup>	8.09 ± 4.93 <sup>a</sup>	15.64 ± 12.32 <sup>a</sup>	12.15 ± 4.11 <sup>a</sup>
Diab+water	125.84 ± 4.34 <sup>b</sup>	123.71 ± 1.61 <sup>b</sup>	123.62 ± 1.99 <sup>c</sup>	19.70 ± 1.64 <sup>a</sup>	68.49 ± 9.86 <sup>a</sup>	91.86 ± 69.19 <sup>b</sup>
Diab+ Met	110.29 ± 6.20 <sup>a</sup>	105.26 ± 2.99 <sup>a</sup>	100.25 ± 2.37 <sup>b</sup>	9.83 ± 0.82 <sup>a</sup>	2.86 ± 2.47 <sup>a</sup>	3.44 ± 0.00 <sup>a</sup>
Diab+ <i>NLME</i>	95.40 ± 2.14 <sup>a</sup>	95.87 ± 1.42 <sup>a</sup>	82.12 ± 14.03 <sup>a</sup>	9.83 ± 2.47 <sup>a</sup>	9.25 ± 8.21 <sup>a</sup>	3.44 ± 1.64 <sup>a</sup>

*NLME*: Namnam Leaves Methanol Extract, Met: Metformin. Diab: Diabetic. Each data is expressed in average ± standard deviation, n=3. Numbers followed with different letters in the same column are significantly different ( $P < 0.05$ ).

**Table 4.** Results of *in vivo* measurement of triglyceride levels.

Group	Triglyceride (mg/dL)		
	H7	H14	H21
Normal	125.33 ± 3.57 <sup>a</sup>	105.27 ± 10.51 <sup>a</sup>	114.24 ± 3.01 <sup>a</sup>
Diab + water	157.20 ± 19.72 <sup>b</sup>	150.26 ± 8.48 <sup>b</sup>	135.84 ± 2.90 <sup>a</sup>
Diab + Met	92.75 ± 34.92 <sup>a</sup>	103.80 ± 7.07 <sup>a</sup>	110.74 ± 6.39 <sup>a</sup>
Diab + <i>NLME</i>	117.93 ± 2.95 <sup>a</sup>	105.93 ± 8.80 <sup>a</sup>	118.45 ± 22.26 <sup>a</sup>

*NLME*: Namnam Leaves Methanol Extract, Met: Metformin. Diab: Diabetic. Each data is expressed in average ± standard deviation, n = 3. The numbers followed by different letters in the same column are significantly different ( $P < 0.05$ ).

**Table 5.** Results of *in vivo* measurement of glycogen levels.

Group	Glycogen (mg)		
	H7	H14	H21
Normal (control)	112.23 ± 1.41 <sup>b</sup>	97.81 ± 3.50 <sup>b</sup>	83.56 ± 7.99 <sup>b</sup>
Diab+water	36.23 ± 21.04 <sup>a</sup>	19.94 ± 2.69 <sup>a</sup>	4.61 ± 3.63 <sup>a</sup>
Diab+ Met	53.40 ± 11.12 <sup>a</sup>	115.84 ± 17.51 <sup>b,c</sup>	82.93 ± 35.72 <sup>b</sup>
Diab+ <i>NLME</i>	113.18 ± 2.83 <sup>b</sup>	112.94 ± 12.48 <sup>b,c</sup>	109.32 ± 23.99 <sup>b</sup>

*NLME*: Namnam Leaves Methanol Extract, Met: Metformin. Diab: Diabetic. Each data is expressed in average ± standard deviation, n = 3. Numbers followed with different letters in the same column are significantly different ( $P < 0.05$ ).

According to Diani and Pulungan [17], metformin is from the biguanide class of oral antidiabetic drugs acting to suppress the production of glucose by the liver and reduce insulin resistance. In this study, the blood glucose level in normal condition (Group 1) is lower, 90.05 – 103.31 mg/dL, compared to that of Group 2 (Diab+water),

123.6 – 125.84 mg/dL (**Table 3**). From the data, it is clear that a high-fat diet and 30% sucrose cause an increase in blood glucose levels. This was confirmed with the Tuckey test showing a significantly different result ( $p < 0.05$ ) (**Table 3**). In Group 2 (Control Diabetic), a high-fat diet alone also resulted in increased blood glucose levels.

The results of insulin analysis for diabetic rats that were treated with only water (Diab) showed an increase in the blood glucose level, and at the same time, the insulin level was also high compared to the other Groups (**Table 3**). However, the high insulin level condition was not able to reduce the blood glucose which presumed the rats had developed insulin resistance. Augustine et al. [13] during the OGT (Oral Glucose Tolerance) test in their study demonstrated that when diabetic rats experienced hyperglycemia, the serum insulin increased concurrently indicating that insulin resistance might have occurred.

Also, when either metformin or Namnam Leaves (*Cynometra cauliflora*) extract was administered. It is presumed that this reduction in insulin concentration is an indicator that both metformin and *NLME* can increase sensitivity to insulin after the resistance. This was also explained in the study of Augustine et al. [13] that the antihyperglycemic activity of *A. paniculate* was also expected from its capability to reduce insulin levels as the result of an increase in insulin sensitivity.

#### **Triacylglycerol/Triglyceride Analysis**

The capability of *NLME* to reduce blood glucose levels is expected from the active compounds found in Namnam plants, i.e. flavonoids and their derivatives. Another study recorded that routine oral administration of flavonoids to diabetic rats caused a reduction in blood plasma glucose levels and an increase in insulin, glycogen content, and hexokinase enzyme activity.

The results of triglyceride analysis showed that the triglyceride concentration in rats in Group 2 (Diab) was in the range of between 135.84 mg/dL to 157.20 mg/dL (**Table 4**). These values were higher than the rats in Group 1 (Normal, without high fat and sucrose induction), with triglyceride concentrations ranging between 105.27 mg/dL and 125,33 mg/dL (**Table 4**). Further, most of the One Way Anova statistical tests showed significant differences in triglyceride levels of every Group ( $p < 0.05$ ). This indicated that a high-fat diet and 30% sucrose (carbohydrate) in the rats in this study had a significant effect on the alteration in triglyceride levels. There is a relationship between lipid profile and the progression of type 2 diabetes mellitus [18]. The study on Diabetic patients found that high levels of total cholesterol (TC), triglycerides (TG), and Low-Density Lipoprotein-

Cholesterol (LDL-C) were found in 47.3%, 76.7%, and 41.3% of patients respectively [19].

Increased triglyceride levels in a high carbohydrate (sucrose) diet will increase fructose-2,6-bisphosphate concentration making phosphofructokinase-1 more active and stimulating glycolysis reaction. This reaction will further cause glucose to be converted into fatty acid. In combination with glycerol, free fatty acid, will form triacylglycerol (TG). Accordingly, similar to a high-fat diet, the more carbohydrate consumed, the higher the concentration of triacylglycerol in the blood.

Shen and Chang [7] induced experimental rats with carbohydrate (fructose) to be T2DM models and found an increase in triacylglycerol, from normal ( $87.5 \pm 11.5$  mg/dL) to  $140.4 \pm 28.0$  mg/dL. According to Wurdianing et al. [20], overconsumption of fat could increase cholesterol, LDL, and triglyceride, and reduce HDL.

The result of this study also showed that the triglyceride level from Day 7 to Day 12 was reduced by 18-41% when the diabetic rats were administered with metformin (Diab+Met) and reduced by 13-30% when administered with Namnam Leaves Methanol Extract (*NLME*). When compared to the condition of the control diabetic (Diab), this result is significant ( $p < 0.05$ ) (**Table 5**). This condition, therefore, demonstrated that both metformin and *NLME* reduce triglyceride levels in diabetes mellitus.

According to Eidi et al. [21], triacylglycerol (TAG) reduction was caused by the restriction of fatty acid synthesis. According to Al-Sulaiti et al. [22] that adipose tissues from obese IR (Insulin Resistant) and T2DM individuals exhibit TAG-specific signatures that may contribute to their increased risk compared to their insulin-sensitive. One way to reduce cholesterol, LDL, and triglyceride is by consuming antioxidant-containing food. The study of Sumarlin et al. [23] showed that Namnam plants (*Cynometra cauliflora*) had antioxidant activity with an  $IC_{50}$  value of 0.0048 mg/mL.

In addition, plasma triglyceride reduction in diabetic rats administered with *NLME* could also be caused by active compounds in the Namnam plants, i.e. flavonoids and their derivatives. Sumarlin et al. [23] stated that *NLME* contained phenolic compounds, flavonoids, vitamins, and  $\beta$ -carotene. GC-MS (Gas Chromatography and Mass Spectroscopy) analysis of *C. cauliflora* leaves detected the presence of phytol, vitamin E, and  $\beta$ -sitosterol. Besides, the phytochemical evaluation of

*C. cauliflora* leaves showed the presence of flavonoids, saponins, and phenolic compounds [6].

Flavonoid treatment reduced triglyceride and cholesterol levels in the blood and increased serum HDL-C levels, as compared with the model group. High-fructose diet administration significantly increased Fatty Acid Synthase mRNA. flavonoids successfully downregulated leptin levels and the majority decreased the relative weights of epididymal adipose tissue. Therefore, flavonoids may function in a similar way to epigallocatechin gallate[24]. The antidiabetic activity of *NLME* corresponds to the metformin drugs, which are not significantly different ( $p < 0.05$ ) (**Table 4**).

### **Glycogen analysis**

Increased plasma glucose causes pancreatic  $\beta$  cell dysfunction, death, and glycogen accumulation in type 2 diabetes [25]. From this study, it was shown that the glycogen level of rat liver in diabetic condition (Diab) was lower than normal, with a reduction ranging between 68-94%, which is significantly different ( $p < 0.05$ ) (**Table 5**). Glycogen reduction in diabetic rats (Diab) indicated some interference in glucose absorption by the liver, or there could be a disturbance in glycogen synthesis. This is also seen in the study of Bhat et al. [26] which showed a decrease in liver glycogen in the diabetic rat group compared to the normal control group with a glycogen content ratio of  $8.33 \pm 0.741$  mg/g and  $23.91 \pm 1.74$  mg/g, respectively.

In the present study, the administration of metformin (Diab+Met) and Namnam leave methanol extract (Diab+*NLME*) could increase the liver glycogen content (**Table 5**). The increase is ranging between 32-94% (Diab+Met) and 68-94% (Diab+*NLME*) compared to control Diabetes (Diab) (**Table 4**). The increased value is significantly different ( $p < 0.05$ ) compared to the control, diabetic (**Table 5**). *NLME* administration gave a higher value than metformin and control, diabetic. Packirisamy et al. [27] reported that the administration of *Coccinia grandis* extract (CGE) showed a significant increase in the levels of glycolytic enzymes and glycogen content and a decrease in the levels of gluconeogenic enzymes in the liver of diabetic-treated rats. The same condition also occurred in the study of Bhat et al., [26] where the liver glycogen content increased when a group of diabetic rats was given an aqueous extract of *Momordica charantia*.

Further, the increase in liver glycogen caused glucose-6-phosphate accumulation, which allosterically hindered the glycogen phosphorylase enzyme [28]. Sumarlin et al., [15], through rat's diaphragm examination, have also proven that the Namnam leaves methanol extract can increase glucose absorption by  $0.4254 \pm 3.23$  mg/mL/30 minutes at *NLME* concentration of 450 mg/dL.

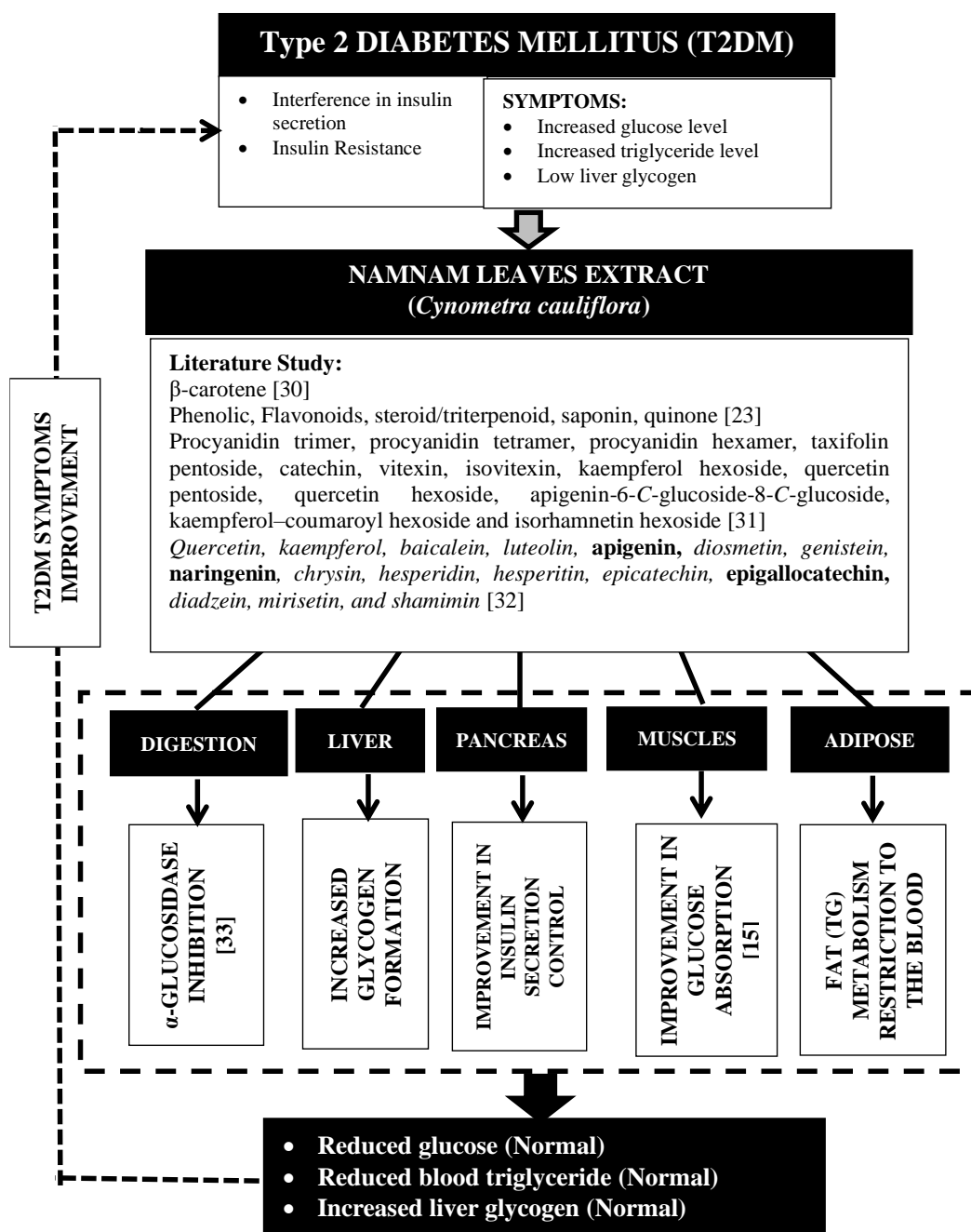
Accordingly, from a series of studies conducted, it may be expected the mechanism of Namnam Leaves (*Cynometra cauliflora*) Methanol Extract (*NLME*) in improving the symptoms of Diabetes Mellitus Type 2 (T2DM). The first symptoms of T2DM are increased blood glucose level, increased triglyceride, and low liver glycogen. These conditions have resulted from alterations in insulin secretion and resistance. *NLME* contains flavonoids,  $\beta$ -carotene, and vitamin C [23]. The flavonoid compound from the methanolic extract of *T. angustifolia* leaves possesses potent anti-diabetic (hypoglycemic) activity in STZ-induced diabetic rats [29].

### **Mechanism of Action of Antidiabetic Activity of Namnam Leaf Extract (*Cynometra Cauliflora*)**

Accordingly, from a series of studies conducted, it may be expected the mechanism of Namnam Leaves (*Cynometra cauliflora*) Methanol Extract (*NLME*) in improving the symptoms of Diabetes Mellitus Type 2 (DMT2) (**Figure 2**). The first symptoms of DMT2 are increased blood glucose level, increased triglyceride, and low liver glycogen. These conditions have resulted from alterations in insulin secretion and resistance. *NLME* contains  $\beta$ -carotene [30], flavonoids, steroid/triterpenoid, saponin, and quinone [23], and various references reported that those compounds and components have antidiabetic activities [31], [32] (**Figure 2**). Secondary metabolites that have antidiabetic activity are flavonoids, saponins, tannins, and quinones which can inhibit the  $\alpha$ -glucosidase enzyme [33]. Supplement saponins have shown that increase  $\beta$ -carotene intake will decrease insulin resistance [34]. This can be seen from the research that after four weeks of saponins supplementation, the disorders in the metabolism of glucose and lipids were significantly weakened, and the insulin resistance of diabetic mice was improved [35]. The results suggest that the saponin of *M. cymbalaria* possesses potential antidiabetic activity with respect to insulin secretion, which may be attributed to modulation of calcium channel, and  $\beta$ -cell rejuvenation [36].

In other studies, the inhibition effects of eight quinones were studied against Aldose Reductase [37]. Aldose reductase (AR) is the first enzyme in the polyol pathway and converts glucose to sorbitol. It plays a vital role as a glucose-reducing agent and is involved in the pathophysiology of diabetic complications [37]. From the results of this study, it is shown that

NLME has the capability to improve DMT2 symptoms in Sprague Dawley rats, which is considered due to various factors:  $\alpha$ -glucosidase enzyme inhibition [38], increased liver glycogen formation, improvement in insulin secretion control, improvement in glucose absorption by the muscles, and fat metabolism restriction to the blood, among others.



**Figure 2.** The Mechanism Hypotetik of Namnam Leaves Methanol Extract (NLME) to improve the DMT2 symptoms.

**CONCLUSION**

This study concludes that Namnam Leaves Methanol Extract (NLME) can lower

blood glucose levels by 23 – 34%, lower triglyceride levels by 13 – 30%, and increase liver glycogen by 68 – 96%. The leaves of the Namnam plant (*Cynometra cauliflora*) have



the activity as antidiabetic, particularly for Diabetes Mellitus Type 2 (T2DM). The mechanism expected to lower blood glucose in those with T2DM is, among others,  $\alpha$ -glucosidase inhibition, increased liver glycogen formation, improvement in insulin secretion control, increased muscle glucose absorption, and fat metabolism restriction to the blood. To establish the validity of these mechanisms, further investigations, such as in vitro and in vivo experiments, should be conducted. Additionally, it would be valuable to explore the molecular pathways and cellular targets involved in these processes.

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