

**Original Article:**

**Syzygium polyanthum protects against diabetic cardiac apoptosis in the chronic diabetes mellitus**

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**Abstract**

**Introduction:** Hyperglycemia has become the main characteristic of diabetes mellitus. Persistent hyperglycemia directly activates cell death pathway that plays pivotal role in the diabetic complication including diabetic cardiomyopathy. Syzygium polyanthum plays a beneficial role in the diabetic condition by reducing the blood glucose concentration, however the role of this natural resources in preventing further complication of diabetes mellitus has not been revealed fully yet. **Method:** Syzygium polyanthum dry extract (300 mg/kg body weight) were given daily for 28 days in the streptozotocin-induced diabetic rat. Measurement of blood glucose concentration were done three times during the study, meanwhile cholesterol concentration, myocardial diameter and cardiac apoptosis were measured on the day 28 of the study. Cardiac apoptosis was analyzed by the TdT-mediated dUTP nick end-labelling (TUNEL Assay). **Results:** Persistent hyperglycemia as well as cardiac apoptosis was significantly observed in the diabetic rat (D) on the day 28 of the study confronted to the normal rat (N). Interestingly, significant blood glucose reduction in concomitant with a lesser concentration of cardiac apoptosis were observed in the diabetic rat received 28 days Syzygium polyanthum extract (DS) confronted to the D rat. Additionally, lower plasma cholesterol concentration was significantly observed in the DS rat confronted to the D rat. **Discussion:** Significant cardiac apoptosis was observed in consistent with persistent hyperglycemia in the D rat as well as lesser cardiac apoptosis was observed in accordance with blood glucose concentration reduction in the DS rat. Therefore, Syzygium polyanthum may play beneficial role in the diabetic-associated cardiac apoptosis through its direct effect on the blood glucose concentration reduction. However further analysis should be done to fully elucidate the apoptotic pathway that involved.

**Keyword:** Diabetes mellitus, Syzygium polyanthum, Apoptosis, Streptozotocin, TUNEL

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**Introduction:**

Diabetes mellitus has become global concern since it affects many patients worldwide. It is associated with either absolute deficiency of insulin secretion or relative deficiency of insulin function from the insulin resistance resulting in the condition called hyperglycemia.<sup>1</sup> Accumulating evidences have shown that persistent chronic hyperglycemia may

lead to several diabetic complication including diabetic cardiomyopathy.<sup>1-3</sup> In brief, persistent hyperglycemia directly activates several cardiac apoptotic pathway at least through the cytochrome c-activated caspase-3 dependent pathway and through the reactive oxygen species (ROS) pathway.<sup>4,5</sup>

Moreover, hyperglycemia have been shown

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to activate endoplasmic reticulum (ER) stress-mediated cardiac apoptosis through the alteration of calcium regulation in the myocyte of diabetic rat.<sup>6-8</sup> Significant increases of cardiac apoptosis may further cause heart dysfunction or failure by reducing the contractility and the pumping function of the heart.<sup>9</sup> Thus, controlling blood glucose concentration still remain the most important strategy in the diabetic mellitus management. *Syzygium polyanthum* is widely used in Indonesia not only as a spice-leaf but also as a medicinal plant for diabetic patients.<sup>10</sup> Phytochemical analysis have shown that *Syzygium polyanthum* leaf is rich in steroid, alkaloid, tannin and flavonoid meanwhile its ripened fruit is rich with saponin. Therefore, *Syzygium polyanthum* is a potential sources of antioxidant and cytotoxic compound.<sup>11</sup> Accumulating evidences have shown further the beneficial role of *Syzygium polyanthum* treatment in many disease including myocardial infarction,<sup>12</sup> hypercholesterolemia,<sup>13</sup> and hypertension.<sup>14</sup> Recently, six days treatment of *Syzygium polyanthum* leaf methanolic extract may reduce hyperglycemia condition by inhibiting the intestinal glucose absorption and the stimulation of glucose uptake.<sup>10</sup> However, the role of 28 days treatment of *Syzygium polyanthum* dry extract in the diabetic cardiac complication have not been fully elucidated yet. This study was done to further evaluate the role of *Syzygium polyanthum* dry extract on the diabetic-associated cardiac apoptosis.

## Methods and Materials:

### *Diabetes induction*

Diabetes was induced using streptozotocin (STZ; Sigma-Aldrich Inc., USA). STZ was dissolved in the citrate buffer with pH 4.5 and administered intraperitoneally in the 8 to 10 weeks male Sprague-Dawley rat using sterile syringe with a single injection (40 mg/kg body weight) as described in the reference.<sup>15</sup> Five days after injection, blood glucose concentration were measured and rat with a blood glucose concentration of higher than 250 mg/dL were decided as diabetic.

### *Experimental group*

Diabetic-confirmed rat were randomly divided into two groups of the diabetic rat without treatment as a positive control (D group; n = 6) and the diabetic group received treatment (DS group; n = 5). The normal rat were divided into two group of the normal rat received solvent as the negative control (N group; n = 5) and the normal rat received treatment (NS group; n= 5). Rat were

allowed to free access of food and water during the research and were treated in accordance with the ethical animal protocol of Faculty of Medicine, Universitas Islam Negeri Syarif Hidayatullah Jakarta and the recommendation of the Declaration of Helsinki.

### *Syzygium polyanthum dry extract treatment*

Dry extract of *Syzygium polyanthum* was received from the Bogor Agricultural University. For the treatment, 300 mg/kg BW of *Syzygium polyanthum* dry extract was dissolved in the distilled water and given every evening for 28 days to the NS and DS group. Dose was decided based on the results of previous publication done by another center.<sup>10</sup>

### *Metabolic parameters*

Measurement of blood glucose concentration was done on the day 1, 14 and 28 of the study from the rat tail using Nescio glucose strip and Nescio glucose meter (Nescio Inc., UK). Total plasma cholesterol concentration was measured on the day 28 of the study by using a total cholesterol kit (Sclavo Diagnostics International, Siena, Italy) in accordance with the company protocol of plasma total cholesterol concentration measurement.

### *Hematoxylin-eosin (HE) staining*

Frozen left ventricle tissue were cut and fixed in the slides using 4% paraformaldehyde. The slides were stained using HE staining. Myocardial diameter was measured by counting the cross-sectional area of cardiomyocytes at the 200x magnification.

### *Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)*

TUNEL staining was performed using an apoptosis assay of In Situ Apoptosis Detection Kit (Takara Bio Inc., USA) in accordance with the company protocol. In brief, the staining processes were deparaffinization, enzymatic process, endogen peroxidase inactivation, labeling process, antigen and antibody process, staining and counterstaining process, and tissue fixation. Stained cell were considered as positive apoptotic cell. To measure cardiac apoptosis, we counted the positive apoptotic cells in fifty fields taken consecutively from three replicates of three samples from each group at the 200x magnification. The result was shown as a percentage of the total apoptotic cells to the total fields counted as described before.<sup>16</sup>

All digital images were photographed using BX-41 microscope and analyzed using DP2-BSW Olympus Software (Olympus Corp., Japan).

### Statistical analysis

Numerical data were shown as means and standard of deviation (SD). Data were analyzed using one way analysis of variance (ANOVA) or Kruskal-Wallis, wherever applicable. Probability value less than 0.05 ( $p < 0.05$ ) was defined as significant.

### Results

#### Rat diabetic model

As depicted in the Table 1, five days after the injection, plasma blood glucose concentrations were increased in the STZ-receiving rat confronted to the normal rat. Blood glucose concentration for more than 250 mg/dl confirmed the animal to be diabetic. Diabetic-confirmed rat were divided in to the diabetic and diabetic with treatment group. Blood glucose concentrations were significantly different in the D group and the DS group confronted to the N group on the day 1 of the study. Furthermore, blood glucose concentration was not significantly different in the D group confronted to the DS group.

#### Role of *Syzygium polyanthum* on the blood glucose concentration

As depicted in Table 1, blood glucose concentration of the N group on the day 1, day 14 and day 28 of the study was  $130.25 \pm 12.28$  mg/dl,  $130.75 \pm 14.45$  mg/dl and  $154.75 \pm 6.28$  mg/dl, consecutively. There were no significant differences of blood glucose concentrations among days of the study inside the N group. Additionally, blood glucose concentration of the NS group was  $132.50 \pm 25.33$  mg/dl,  $135.75 \pm 16.58$  mg/dl and  $136.50 \pm 35.37$  mg/dl on the day 1, day 14 and day 28 of the study, consecutively. There were no significant differences observed among days of the study inside the NS group. Moreover, blood glucose concentrations were not significantly different between the N and the NS group. These results have shown us that *Syzygium polyanthum* did not possess hypoglycemic activities in the normal condition. Additionally, 28 days *Syzygium polyanthum* treatment did not alter blood glucose concentration and did not elicit further hypoglycemia during the long term decoction of the study in the normal rat (Fig. 1).

Blood glucose concentration was significantly high in the diabetic rat as shown by the concentration of  $598.00 \pm 4.47$  mg/dl ( $p < 0.01$ ) and it remained high on the day 14 and 28 of the study as shown by the blood glucose concentrations of  $551.60 \pm 70.26$  mg/dl and  $600.00 \pm 0.00$  mg/dl, respectively (Table 1). Once daily *Syzygium polyanthum* dry extract treatment started to reduce blood glucose

concentrations on the day 14 of the study, however, no significant difference was observed confronted to the diabetic group (Fig. 1). Significant reduction of blood glucose concentration were observed on the day 28 of the treatment confronted to the D group. Conclusively, daily treatment of *Syzygium polyanthum* extract may play a significant role on the reduction of blood glucose concentration in the diabetic condition only after 28 days of the treatment.

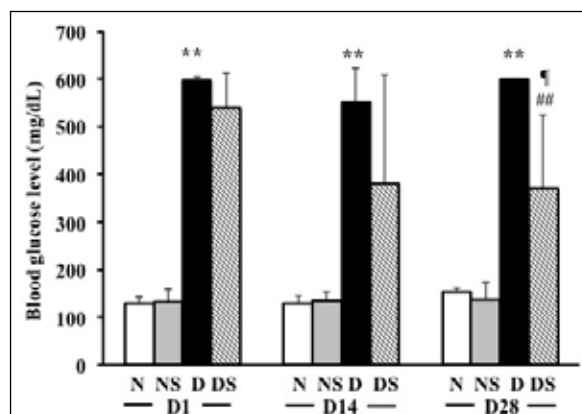


Figure 1. Blood glucose concentration among groups during the study. N = normal rat; NS = normal rat with extract; D = diabetic rat; DS = diabetic rat with extract. \*\* $p < 0.01$  vs the normal rat; ## $p < 0.01$  vs. the diabetic rat; ¶ $p < 0.05$  vs. the diabetic with extract on the day 1 group

Total cholesterol concentrations were measured on the day 28 of the study. As shown in the Table 1, plasma total cholesterol concentration were  $176.35 \pm 15.56$  mg/dl,  $179.25 \pm 5.92$  mg/dl,  $202.24 \pm 24.54$  mg/dl and  $131.05 \pm 32.63$  mg/dl in the N, NS, D and the DS group, consecutively. Significant difference was observed in the D

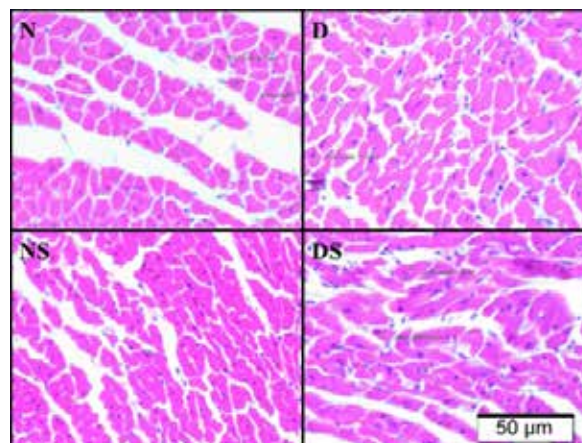


Figure 2. Myocardial diameters stained with Hematoxylin-Eosin (HE) and analyzed from the 200x magnification. N = normal rat; NS = normal rat received treatment; D = diabetic rat; DS = diabetic rat received treatment.

confronted to the DS group, suggesting a result that *Syzygium polyanthum* dry extract treatment may play a beneficial role in the cholesterol regulation of the diabetic rat.

#### Myocardial diameters

Myocardial diameters were analyzed on the day 28 of the study. In brief, myocardial diameters were  $31.56 \pm 0.06$   $\mu\text{m}$ ,  $32.50 \pm 0.09$   $\mu\text{m}$ ,  $26.16 \pm 1.24$   $\mu\text{m}$  and  $32.94 \pm 3.31$   $\mu\text{m}$  in the N, NS, D and DS group, consecutively (Table 1). A slight reduction of myocardial diameter was observed in the D group confronted to other group. However, the reduction did not attain significance in the statistical analysis (Fig.2).

#### Cardiac apoptosis

As depicted in Fig. 3, the percentages of the cardiac apoptosis were  $6.33 \pm 0.58$ ,  $4.78 \pm 4.20$ ,  $34.41 \pm 4.50$  and  $22.75 \pm 2.87$  in the N, NS, D and

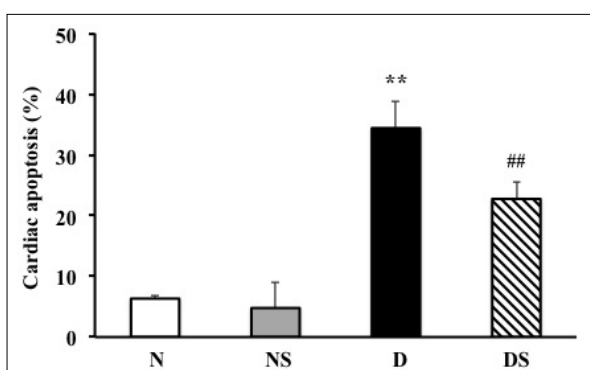
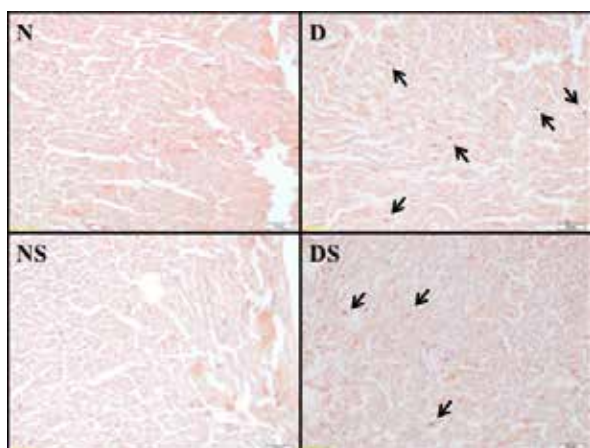


Figure 3. Cardiac apoptosis stained with TUNEL assay and analyzed from the 200x magnification. N = normal rat; NS = normal rat with extract; D = diabetic rat; DS = diabetic rat with extract. \*\*p<0.01 vs the normal rat; ##p<0.01 vs. the diabetic rat.

DS group, consecutively. In concomitant with hyperglycemia condition, significant level of cardiac apoptosis was noticed in the diabetic group confronted to the normal group (Fig. 3, Table 1). Furthermore, 28 days daily treatment of *Syzygium*

polyanthum significantly reduce cardiac apoptosis in accordance with the significant decrease of the blood glucose concentration (Fig. 3, Table 1). Our result have shown that high concentration of cardiac apoptosis was observed in concomitant with the persistent hyperglycemia on the day 28 of the study in the diabetic group.

**Table1:** Metabolic parameters among the groups

| Metabolic parameters                | 1 <sup>st</sup> day | 14 <sup>th</sup> day | 28 <sup>th</sup> day          |
|-------------------------------------|---------------------|----------------------|-------------------------------|
| Blood glucose concentration (mg/dl) |                     |                      |                               |
| N                                   | 130.25±12.28        | 130.75±14.45         | 154.75±6.24                   |
| NS                                  | 132.50±25.33        | 135.75±16.58         | 136.50±35.37                  |
| D                                   | 598.00±4.47**       | 551.60±70.26**       | 600.00±0.00**                 |
| DS                                  | 538.67±71.38        | 380.83±227.18        | 369.17±154.42 <sup>##,†</sup> |
| Cholesterol concentration (mg/dl)   |                     |                      |                               |
| N                                   |                     |                      | 176.35±15.56                  |
| NS                                  |                     |                      | 179.25±5.92                   |
| D                                   |                     |                      | 202.24±24.54                  |
| DS                                  |                     |                      | 131.05±32.63 <sup>##</sup>    |
| Myocardial diameter (um)            |                     |                      |                               |
| N                                   |                     |                      | 31.56±0.06                    |
| NS                                  |                     |                      | 32.50±0.09                    |
| D                                   |                     |                      | 26.14±1.24                    |
| DS                                  |                     |                      | 32.94±3.31                    |

N = normal rat; NS = normal rat with extract; D = diabetic rat; DS = diabetic rat with extract. \*\*p<0.01 vs. the normal group; <sup>##</sup>p<0.01 vs. the diabetic group and <sup>†</sup>p<0.05 vs. the diabetic with extract on the day 1.

Additionally, significant reduction of cardiac apoptosis was observed in the DS group in consistent with the significant blood glucose concentration reduction after 28 days of treatment. These result strongly suggest that *Syzygium polyanthum* may play significant role in the diabetic-associated cardiac apoptosis probably by controlling the blood glucose concentration. However, further analysis of which apoptotic pathway that involved should be done to fully elucidate the mechanism.

#### Discussion:

Several important findings from the study were : (1) Significant hyperglycemia was observed on the day 28 of the study along with a significant concentration of cardiac apoptosis in the diabetic rat; (2) Significant lesser concentration of cardiac apoptosis was observed in concomitant with the reduction of blood glucose concentration in the diabetic rat received 28 days *Syzygium polyanthum*

dry extract treatment. Persistent hyperglycemia due to absolute or relative deficiency of insulin in the diabetic mellitus have been proposed as a culprit of many diabetic complication including diabetic cardiomyopathy. Hyperglycemia have been proposed to elicit cardiac dysfunction and failure by directly activating the caspase-3-dependent,<sup>4</sup> ROS-dependent<sup>5</sup> and ER stress-mediated apoptotic pathway in the heart.<sup>7</sup> Thus, controlling blood glucose concentration still remain the most important strategy in the diabetic complication prevention. *Syzygium polyanthum* has been shown to give beneficial effect in the diabetic condition.<sup>10</sup> In brief, six days of *Syzygium polyanthum* treatment has been shown to reduce fasting blood glucose through the inhibition of intestinal glucose absorption and the stimulation of glucose uptake in the muscle.<sup>10</sup> Additionally, 8 weeks supplementation of *Syzygium polyanthum* and *Andrographis paniculata* extract mixture along with the 1000 mg metformin treatment significantly reduce fasting blood glucose and postprandial glucose concentration in the type-2 diabetic patients in one randomized clinical trial.<sup>17</sup> However, limited evidences have reported the role of *Syzygium polyanthum* in the prevention of diabetic-related cardiac complication. We have shown that persistent hyperglycemia appeared on the day 1, day 14 and day 28 of the study in the diabetic rat when confronted to the normal rat. In concomitant with the highest blood glucose concentration on the day 28 of the study, we also found significant level of cardiac apoptosis in the diabetic rat. Our result strongly support the previous evidence regarding the correlation of hyperglycemia and the cardiac apoptosis.<sup>4</sup> Interestingly, 28 days daily treatment of 300 mg/kg BW *Syzygium polyanthum* dry extract significantly reduced blood glucose concentration in the diabetic rat. We further found a lesser cardiac

apoptosis in the diabetic rat received 28 days *Syzygium polyanthum* treatment when confronted to the diabetic rat. How *Syzygium polyanthum* reduce the cardiac apoptosis concentration may be related to its activity as an anti-oxidant that inhibits the ROS-mediated apoptotic pathway<sup>11,13,17,18</sup> or as a hypoglycemic agent that mimic acarbose effect by directly inhibits the intestinal glucose absorption.<sup>10,18</sup> Our result support the importance of controlling hyperglycemia in the prevention of cardiac-related diabetic complication by inhibiting cardiac apoptosis. To the extent of our knowledge, this is the first study to report the role of *Syzygium polyanthum* extract treatment in the prevention of cardiac-related diabetic complication at least in part through the inhibition of cardiac apoptosis. Further analysis are still required to fully elucidate the role of *Syzygium polyanthum* in the various apoptotic pathway involved in the development of diabetic cardiac complication.

#### **Limitations and Problems:**

The lack of apoptotic signaling pathway analysis and the various doses given to the rat became the main limitations of this study.

#### **Ethical Approval:**

This research was approved by the Ethics Committee of Universitas Islam Negeri Syarif Hidayatullah Jakarta

**Conflict of interest:** None declared

#### **Acknowledgement:**

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#### **Author's Contributions:**

Data gathering and idea owner of this study: FRS, HH

Study design: FRS

Data analysis and consultation: FRS, HH, FA, IN, AR, FS, N, PRA

Writing and submitting manuscript: FRS



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