Antihyperglicemic effectiveness comparison of ethanol extract of soursop leaf (Annona muricata L.) against acarbose in streptozotocin-induced diabetic white rats

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Abstract. Type 2 diabetes mellitus is major health problem worldwide, including in Indonesia. Acarbose, an alpha glucosidase inhibitor, is one of oral pharmacologic management commonly used in clinical practice with potent side effect in long-term use. Flavonoid-and-tannins containing soursop leaf can stimulate insulin secretion and repair beta pancreatic cell dysfunction. This study was aimed to investigate the comparison on antihyperglycemic effect between ethanol extract of soursop leaf (Annona muricata L.) and acarbose in streptozotocin-induced diabetic white rats. This study was experimental research with pre- and post-test control group design enrolling 30 hyperglycemic white male rats. They were divided into 2 control groups (C(+) was given by aquades and C(-) was given by acarbose) and 3 treatment groups (P1 was given 10 mg/200gBW extract, P2 was given 20 mg/200gBW extract, and P3 was given 30 mg/200gBW extract). Data analysis was using paired-T test. This study showed that there is a significant decrease in blood glucose levels in P1, P2, and P3 (95.08 mg/dL, 113.31 mg/dL, and 125.17 mg/dL; p<0.05). Ethanol extract of soursop leaf has antihyperglycemic effect in dose-dependent response. The 30 mg of ethanol extract of soursop leaf is not inferior to 1.8 mg of acarbose in antihyperglycemic effect.

1. Introduction
Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease or disorder characterized by high blood sugar levels accompanied by impaired metabolism of carbohydrates, lipids, and proteins as a result of insulin function insufficiency[1]. Wild, et al. (2004) reported that T2DM in Indonesia in 2000 reached 8.4 million people. Further, the number of people with T2DM in Indonesia is ranked 4th after India, China, and the United States.1 One of the risk factors for DM is obesity due to changes in people’s lifestyle which tend to consume foods with high in fat and fructose without adequate physical activity [2].

Pharmacologic treatment is a part of management of T2DM after dietary approach and establishing appropriate physical activity. Oral antidiabetic agents are more favorable pharmacologic treatment than insulin, but they provide undesirable side effects. Traditional plant-extracting medicine with favorable safety and tolerability profile is on high demand in the concept of patient-centered approach in managing T2DM [3].

One of the plants that has antidiabetic activity is soursop (Annona muricata L.). The part of the soursop plant that has anti-diabetic properties is the leaves. Based on the results of previous studies showed that administration of ethanolic extract of soursop leaves (Annona muricata L.) affected blood
glucose levels and histology of rat pancreas (*Rattus norvegicus*) induced by *Streptozotocin* [4]. Based on them, we conducted a study to investigate the comparison of the antihyperglycemic effect between ethanolic extract of soursop leaves (*Annona muricata* L.) and acarbose in diabetic male white rats (*Rattus norvegicus*) induced by *streptozotocin*.

2. Materials and Methods

This study was an experimental study with a pre- and post-test design with control group enrolling 30 male white rats (*Rattus norvegicus*). There were 5 groups in this study, namely 2 control groups and 3 treatment groups. The sampling method used in this study was simple random sampling.

The dose of *streptozotocin* administered was 45 mg/200gr of mice, calculated based on the average weight of mice and intraperitoneally injected [1]. Acarbose dose for humans is 100 mg/day. Based on the Laurence and Bacharach conversion table, the conversion of Acarbose dose to humans with body weight (70 kg) to white rats weighing 200 grams is 0.018. So, the dose used in the study was 100 mg x 0.018 = 1.8 mg/200gr [1]. The dose of ethanol extract of soursop leaves (*Annona muricata*) in rats was 50 mg/kg daily, 100 mg/kg daily, and 150 mg/kg daily. 5 for this study through the Laurence and Bacharach formula, the dosage used in rats was for treatment 1 10 mg/200gr, treatment 2 20 mg/200gr, and treatment 3 30 mg/200gr. White rats were divided into 5 groups consisting of negative control groups, namely groups that were only given water and standard feed. Positive control group was the group given Acarbose. Treatment group 1 was the group given soursop leaf ethanol extract at a dose of 10 mg/200gr. Treatment group 2 was the group given soursop leaf ethanol extract at a dose of 20 mg/200gr, and treatment 3 was the group given soursop leaf ethanol extract at a dose of 30 mg/200gr. The next step was that white male Wistar strain rats that had undergone an adaptation period were given standard feed and drinks were ad libitum for 1 week. Furthermore, the mice were induced with *Streptozotocin* and blood glucose levels were measured before being treated. Blood collection was done on the orbital sinus and for measurement using a glucometer. Then, each group received treatment according to the doses that had been determined for 1 week and then examined the blood glucose levels of the white rats of each group after being treated.

All data obtained were tested for normality in advance with the Shapiro-Wilk test because the sample used in this study amounted to 25 tails (<50), if a normal data distribution was obtained, the test could be continued with a data homogenity test performed by the Levene test. The analysis was the parametric difference test to see the difference in the mean values between the 2 groups paired with paired T Test. The parametric analysis test was then performed using the One Way ANOVA test for measurements in paired samples at a 95% confidence level. Statistical tests in this study used the Statistical Product and Service Solutions (SPSS) program.

3. Results and Discussion

The highest average blood glucose level of pre-test was in the positive control group which was 267.82 mg/dL and the smallest average blood glucose level pre-test was found in the treatment group 3 ethanol extract 30 mg/200gr which was 265.15 mg/dL. The highest average post-test blood glucose level was in the negative control group at 269.59 mg/dL and the lowest post-test blood glucose level was found in the positive control at 132.59 mg/dL, as can be seen in Table 1.

The average change in blood glucose levels in the negative control group was -1.95 mg/dL, meaning that the blood glucose of rats increased. The average of the largest changes were in the positive control group of 135.11 mg/dL and the average changes were in the treatment group 1 of the 10 mg/200gr soursop leaf ethanol extract of 95.08 mg/dL, as can be seen in Table 2. Data on the results of blood glucose levels were tested for normality in each group using the Shapiro-Wilk normality test because the data <50 samples. It is normally distributed if the significance value is above 0.05 (p> 0.05). The research data was tested for normality through the Shapiro-Wilk test showing normality of distribution, ie the significance value exceeded 0.05 for all groups of research
data, meaning that all data were normally distributed as in Table 3.

To conduct the One Way Anova test, the research data must be homogeneous. Through the Levene Test test described in Table 4 there is a significant result of > 0.05 for all data groups, so the entire data group is homogeneous.

Analysis of the effect of giving soursop leaf ethanol extract in reducing blood glucose levels was tested based on the average blood glucose between before and after giving treatment to each group. The significance analysis results with paired T-test and said to be meaningful if p <0.05.

| Group                  | Dose of Acarbose | Dose of extract | Blood Glucose | Average       |
|------------------------|------------------|-----------------|---------------|---------------|
| Negative control       |                  |                 | Pretest | Posttest | Pretest | Posttest |
| K1.1                   |                  |                 | 263.87    | 265.64    | 267.64   | 269.59   |
| K1.2                   |                  |                 | 275.55    | 278.35    | 271.17   | 276.74   |
| K1.3                   |                  |                 | 271.17    | 272.51    | 266.06   | 269.42   |
| K1.4                   |                  |                 | 266.06    | 269.42    | 262.77   | 264.60   |
| K1.5                   |                  |                 | 262.77    | 264.60    | 266.42   | 267.01   |
| K1.6                   |                  |                 | 266.42    | 267.01    | 267.64   | 269.59   |
| Positive control       |                  |                 |           |           | 267.82   | 132.59   |
| K2.1                   | 1.78             |                 | 274.09    | 130.58    | 267.82   | 132.59   |
| K2.2                   | 1.79             |                 | 269.71    | 127.84    | 267.82   | 132.59   |
| K2.3                   | 1.74             |                 | 263.14    | 132.30    | 267.82   | 132.59   |
| K2.4                   | 1.68             |                 | 267.88    | 133.33    | 267.82   | 132.59   |
| K2.5                   | 1.67             |                 | 262.41    | 134.36    | 267.82   | 132.59   |
| K2.6                   | 1.79             |                 | 268.98    | 137.11    | 267.82   | 132.59   |
| Treatment 1 of soursop leaf ethanol extract 10 mg/200gr | 10.05 | 274.09 | 177.32 | 267.70 | 172.62 |
| P1.1                   |                  |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P1.2                   | 9.60             |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P1.3                   | 9.45             |                 | 263.14    | 169.76    | 267.70   | 172.62   |
| P1.4                   | 9.15             |                 | 267.88    | 171.48    | 267.70   | 172.62   |
| P1.5                   | 9.90             |                 | 262.41    | 168.38    | 267.70   | 172.62   |
| P1.6                   | 10.15            |                 | 268.98    | 173.88    | 267.70   | 172.62   |
| Treatment 2 of soursop leaf ethanol extract 20 mg/200gr | 19.10 | 264.60 | 151.89 | 266.12 | 152.81 |
| P2.1                   |                  |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P2.2                   | 19.00            |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P2.3                   | 18.70            |                 | 263.14    | 169.76    | 267.70   | 172.62   |
| P2.4                   | 18.40            |                 | 267.88    | 171.48    | 267.70   | 172.62   |
| P2.5                   | 18.80            |                 | 262.41    | 168.38    | 267.70   | 172.62   |
| P2.6                   | 19.00            |                 | 268.98    | 173.88    | 267.70   | 172.62   |
| Treatment 3 of soursop leaf ethanol extract 30 mg/200gr | 28.35 | 267.52 | 142.61 | 265.15 | 139.98 |
| P3.1                   |                  |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P3.2                   | 28.80            |                 | 269.71    | 174.91    | 267.70   | 172.62   |
| P3.3                   | 28.20            |                 | 263.14    | 169.76    | 267.70   | 172.62   |
| P3.4                   | 27.45            |                 | 267.88    | 171.48    | 267.70   | 172.62   |
| P3.5                   | 28.05            |                 | 262.41    | 168.38    | 267.70   | 172.62   |
| P3.6                   | 28.65            |                 | 268.98    | 173.88    | 267.70   | 172.62   |

The results of significance with paired T test showed that the treatment group 1, treatment group 2, treatment group 3 and the positive control group obtained p = .000, while the negative control group showed the results of p = .005, which means showing significant changes as can be seen in Table 5.

Comparison of the effect of dosing on blood glucose levels before being given treatment was then tested for significance by One Way ANOVA test. The results of One Way ANOVA analysis were significant if the value of p <0.05.

The average blood glucose level of pre-test in the negative control group was 267.64 mg/dL, the positive control group was 267.82 mg/dL, the treatment group 1 was 267.70 mg/dL, the treatment group 2 was 266.11 mg/dL, and treatment group 3 was 265.14 mg/dL. The average results were found in groups that were not significantly different because p value = 0.87 (p > 0.05). Tests of blood glucose levels in the pre-test were described in Table 6. The average post-test blood glucose level in the negative control group was 269.58 mg/dL, the positive control group was 132.58 mg/dL, the treatment
group 1 was 172.62 mg/dL, the treatment group 2 was equal to 152.80 mg/dL, and treatment group 3 was 139.97 mg/dL. The average results there are groups that are significantly different because the value of $p = 0.000$ ($p < 0.05$), as can be seen in Table 7.

| Group                  | Blood Glucose | Change | Average |
|------------------------|---------------|--------|---------|
| Negative control       |               |        |         |
| K1.1                   | 263.87        | 265.64 | -1.77   |
| K1.2                   | 275.55        | 278.35 | -2.8    |
| K1.3                   | 271.17        | 272.51 | -1.34   |
| K1.4                   | 266.06        | 269.42 | -3.36   |
| K1.5                   | 262.77        | 264.60 | -1.83   |
| K1.6                   | 266.42        | 267.01 | -0.59   |
| Positive control       |               |        | 135.11  |
| K2.1                   | 274.09        | 130.58 | 143.51  |
| K2.2                   | 269.71        | 127.84 | 141.87  |
| K2.3                   | 263.14        | 132.30 | 130.84  |
| K2.4                   | 267.88        | 133.33 | 134.55  |
| K2.5                   | 262.41        | 134.36 | 128.05  |
| K2.6                   | 268.98        | 137.11 | 131.87  |
| Treatment 1            |               |        | 95.08   |
| of soursop leaf ethanol extract 10 mg/200grBB | | |
| P1.1                   | 274.09        | 177.32 | 96.77   |
| P1.2                   | 269.71        | 174.91 | 94.8    |
| P1.3                   | 263.14        | 169.76 | 93.38   |
| P1.4                   | 267.88        | 171.48 | 96.4    |
| P1.5                   | 262.41        | 168.38 | 94.03   |
| P1.6                   | 268.98        | 173.88 | 95.1    |
| Treatment 2            |               |        | 113.31  |
| of soursop leaf ethanol extract 20 mg/200grBB | | |
| P2.1                   | 264.60        | 151.89 | 112.71  |
| P2.2                   | 259.12        | 147.77 | 111.35  |
| P2.3                   | 262.04        | 147.42 | 114.62  |
| P2.4                   | 271.17        | 154.64 | 116.53  |
| P2.5                   | 277.74        | 162.20 | 115.54  |
| P2.6                   | 262.04        | 152.92 | 109.12  |
| Treatment 3            |               |        | 125.17  |
| of soursop leaf ethanol extract 30 mg/200grBB | | |
| P3.1                   | 267.52        | 142.61 | 124.91  |
| P3.2                   | 259.12        | 137.11 | 122.01  |
| P3.3                   | 265.33        | 138.83 | 126.50  |
| P3.4                   | 266.06        | 140.89 | 125.17  |
| P3.5                   | 271.17        | 143.99 | 127.18  |
| P3.6                   | 261.68        | 136.43 | 125.25  |

| Statistic | Df | Sig. |
|-----------|----|------|
| Shapiro-Wilk |     |      |
| Pre-test  |     |      |
| Negative control | .907 | 6 | .416 |
| Positive control | .975 | 6 | .922 |
| Treatment 1 | .937 | 6 | .636 |
| Treatment 2 | .889 | 6 | .312 |
| Treatment 3 | .980 | 6 | .953 |
| Post-test |     |      |
| Negative control | .911 | 6 | .440 |
| Positive control  | .996 | 6 | .999 |
| Treatment 1 | .973 | 6 | .911 |
| Treatment 2 | .904 | 6 | .399 |
| Treatment 3 | .943 | 6 | .687 |
This study showed a significant change in blood pressure in the positive group, namely the group given acarbose and the treatment groups. We assumed that soursop leaves have antihyperglycemic compounds that are flavonoid, alkaloids, and tannins. The antihyperglycemic effect was assessed by evaluating the significant changes between pre-test blood glucose and post-test blood glucose. The higher the value of change, the lower the blood glucose level, the better the effectiveness. Based on the results in Table 2, the highest antihyperglycemic effect was treatment group 3 (30 mg/200gr of soursop leaf extract) and it was similar with acarbose group in reducing blood glucose. The antihyperglycemic effect of soursop leaf ethanol extract was supported by several studies. Annisa Ratya (2014) showed that soursop leaves contain tannin compounds that can be hydrolyzed divided into gallatanin and ellagitanin which have properties similar to insulin, and flavonoids, especially quercetin, which stimulate insulin secretion and repair damage to pancreatic β cells [5].

Antidiabetic activity itself is found in flavonoids in herbal plants. Tannins and several components of polyphenols, flavonoids, saponins and others affect hypoglycemic, anti-inflammatory, hypotensive and other pharmacological effects. Soursop leaves are known to contain ellagic acid, tannins, flavonoids, polyphenol components, β-cystosterol, and ketekin, but only particular components, such as tannins, flavonoids, and alkaloids, have the properties in repairing pancreatic tissue in patients with DM [6]. Soursop leaves have flavonoid derivatives namely quercetin. Quercetin compounds have the potential as hypoglycemic agent because quercetin and chrysin, which are part of flavonoids at high doses, prevent an increase in blood glucose values due to its stimulating β cells to produce more insulin [6].

Flavonoids have a hypoglycemic effect by several mechanisms, those are inhibiting glucose absorption, increasing glucose tolerance, stimulating insulin release or acting like insulin, increasing glucose uptake by peripheral tissues, and regulating enzymes that play a role in carbohydrate metabolism. In vitro studies also show that quercetin has the potential as an inhibitor of glucose transport by intestinal glucose transporter 2 (GLUT2) and glucose transporter 5 (GLUT5) which are responsible for the absorption of glucose in the small intestine [7].

Tannins are known to stimulate the metabolism of glucose and fat. In addition, tannins have antioxidant activity and inhibit tumor growth. Tannins also have hypoglycemic ability by increasing glycogenesis. This compound has a function as astringent or chelating which can shrink the small intestinal epithelial membrane so as to reduce the absorption of food essence as a result of inhibiting sugar intake and the rate of increase in blood sugar is not too high [8]. Tannins reduce blood glucose levels by increasing glucose uptake through activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase. Hydrolyzed tannins are divided into gallotanin and ellagitanin. Gallotanin can increase glucose uptake while inhibiting adipogenesis. The ellagitanin derivatives on
the other hand, namely lagerstroemin, flosin B, and reginin A have properties similar to insulin (insulin-like compound). These three compounds can increase glucose transport activity into adipose cells \textit{in vitro} [9].

Tabel 6. The pre-test blood glucose levels

| Group              | Average of pre-test blood glucose | Sig. |
|--------------------|-----------------------------------|------|
| Negative control   | 267.64                            |      |
| Positive control   | 267.82                            |      |
| Treatment 1        | 267.70                            | .870 |
| Treatment 2        | 266.11                            |      |
| Treatment 3        | 265.14                            |      |

Tabel 7. The post-test blood glucose levels

| Group              | Average of post-test blood glucose | Sig. |
|--------------------|-----------------------------------|------|
| Negative control   | 269.58                            |      |
| Positive control   | 132.58                            |      |
| Treatment 1        | 172.62                            | .000 |
| Treatment 2        | 152.80                            |      |
| Treatment 3        | 139.97                            |      |

Alkaloids reduce blood glucose by inhibiting glucose absorption in the intestine, increasing the transport of glucose in the blood, stimulating glycogen synthesis and inhibiting glucose synthesis by inhibiting the enzyme glucose 6-phosphatase, fructose 1,6-bisphosphatase, and increasing glucose oxidation through glucose 6-phosphate dehydrogenase. Glucose 6-phosphatase and fructose 1,6-bisphosphatase are enzymes enrolled in gluconeogenesis. Inhibition of these two enzymes reduce the formation of glucose from other substrates other than carbohydrates [10].

4. Conclusions
Ethanol extract of soursop leaf has antihyperglycemic effect in dose-dependent response. The 30 mg of ethanol extract of soursop leaf is not inferior to 1.8 mg of acarbose in antihyperglycemic effect. Further studies are needed to develop soursop leaf as an alternative therapy in managing T2DM.

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