

# 2022 The effect of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract on blood glucose, lipid profiles, and weight loss in alloxan-induced diabetic rats

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# The effect of breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract on blood glucose, lipid profiles, and weight loss in alloxan-induced diabetic rats

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## ABSTRACT

Diabetes mellitus is associated with abnormalities in lipid metabolism and weight loss. This study aimed to examine breadfruit (*Artocarpus altilis* (Parkinson) Fosberg) leaf extract's effects on lipid profiles and weight loss in alloxan-induced diabetic rats. Forty-five male Wistar rats were injected with alloxan and divided into treatment groups: placebo, *Artocarpus altilis* leaf extract (100, 200, or 400 mg/kg) or insulin (6U/200 g). Five additional rats were included as normal controls. Following 14 days of treatments, *Artocarpus altilis* extract lowered the blood glucose (BG) level, but only significant at 400 mg/kg dose. Eighty percent of rats in the placebo group had a significant weight loss compared to 40% of rats in the 400 mg/kg group. The placebo group had significantly higher total cholesterol (TC) compared to controls ( $p < 0.05$ ) and the *Artocarpus altilis* extract treatment significantly reduced the TC level ( $p < 0.05$ ). In conclusion, *Artocarpus altilis* extract treatment improves BG, lipid metabolism, and weight loss in alloxan-induced diabetic rats.

**Keywords:** *Artocarpus altilis*, breadfruit, alloxan, dyslipidemia, weight loss

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## INTRODUCTION

Diabetes mellitus (DM) is a condition characterized by hyperglycemia due to insulin resistance. It is estimated that people with diabetes mellitus have reached 463 million worldwide and are predicted to rise to 578 million by 2030<sup>1</sup>. In addition to hyperglycemia, diabetes mellitus is associated with dyslipidemia due to insulin modulation on lipid metabolism, which subsequently alters the activities or the transport of lipid metabolism enzymes. The impact of diabetic-induced dyslipidemia includes vascular complications, which lead to increased comorbidity in DM patients<sup>2</sup>.

Weight loss is one of the clinical symptoms of non-insulin or insulin-dependent DM. In the absence of insulin, the transport of blood glucose into the cells is averted. As the cells fail to receive glucose as the primary energy source, the body stimulates excessive mobilization of fat from the adipose tissues<sup>3</sup>, leading to diabetic weight loss. There is evidence that diabetic patients who lost weight without having a lifestyle change had increased risks of mortality compared to those who did not lose weight<sup>4</sup>.

The use of herbal products and supplements has increased rapidly over the past three decades, with no less than 80% of people relying on herbal products<sup>5</sup>. It has been reported that more than 1200 traditional plants may have been used as the folklore of antidiabetic treatments<sup>6</sup>. *Artocarpus altilis* (Parkinson) Fosberg or breadfruit is one of the plants that have been empirically used for DM treatments and lipid disorders. In animal studies, *Artocarpus altilis* leaf extract was found to increase pancreatic beta cell number in streptozotocin-induced diabetic rats<sup>7</sup>. In line with this, *Artocarpus altilis* leaf extract also improves Langerhans islands and exocrine tissue structures in the pancreas of alloxan-induced diabetic rats<sup>8,9</sup>. It is believed that *Artocarpus altilis* roles are not limited to glucose control since *Artocarpus altilis* leaf extract was also beneficial to reduce free fatty acid levels in obese rats<sup>10</sup>. Therefore, this study aimed to examine the effect of *Artocarpus altilis* leaf extract administration on lipid profiles and weight loss in diabetic rats induced by alloxan injection. The lipid profiles examined include cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels.

## METHODOLOGY

### Preparation and extraction of *Artocarpus altilis*

*Artocarpus altilis* leaves were harvested in Gowa, South Sulawesi. Only leaves with yellowish color were handpicked from the trees based on the empirical use of *Artocarpus altilis* leaves as a diabetes treatment in the area. The *Artocarpus*

<sup>2</sup>  
*altilis* leaves were authenticated by Dr. A. Mu'Nisa from the Department of Biology, State University of Makassar, Indonesia, with an authentication number of 096/SKAP/LAB.BIOLOGI/VII/2019. The herbarium specimen was collected and deposited in the Laboratory of Pharmacognosy, Hasanuddin University, Indonesia. The leaves were cleaned with tap water, sorted, dried, and finely chopped. *Artocarpus altilis* leaves (150 g) were macerated using 70% ethanol (2.5 L) for three days with an occasional stirring. The result of maceration was evaporated using a rotary vacuum evaporator at 175 mbar coupled with a water bath at 40°C until thick extract was obtained. The remaining solvent was further reduced using a desiccator.

### Chemical preparation

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Alloxan monohydrate was purchased from Sigma Aldrich. Diagnostic kits for cholesterol, triglycerides, LDL, and HDL were purchased from Human Diagnostics Worldwide (Germany).

### Animal preparation

<sup>2</sup>  
Fifty male Wistar rats were purchased from an animal laboratory breeding facility (UD. Wistar, Yogyakarta, Indonesia). Animals were accustomed to the laboratory environment for 14 days before starting the experiment. Animals received standard food and water ad libitum.

### Experimental procedures

<sup>18</sup>  
Forty-five rats were intraperitoneally injected with alloxan at a dose of 155 mg/kg. The alloxan dose was chosen based on a previous study<sup>11</sup> and adjusted in our preliminary study. Ten minutes after injection, rats were given 5% glucose (2 ml) through oral gavage to prevent hypoglycemia in rats. The blood glucose levels were checked daily with a glucometer. After three days post-alloxan injection, only rats with blood glucose levels > 200 mg/dl were defined as diabetic and received treatments according to their groups.

Group I was given a placebo (sodium carboxyl methylcellulose, Na CMC 1%, n=5); group II was given *Artocarpus altilis* leaf extract at the dose of 100 mg/kg; group III was given *Artocarpus altilis* leaf extract at the dose of 200 mg/kg; group IV was given *Artocarpus altilis* leaf extract at the dose of 400 mg/kg; group V received insulin injection at the dose 6 IU/200 g. An additional group of rats (n=5) that did not receive alloxan injection was also involved as normal controls.

Rats were weighed every day before receiving treatments to adjust the dose accordingly. The *Artocarpus altilis* extract treatments, as well as insulin injections, were done once daily for 14 consecutive days. Blood samples were withdrawn at

the end of experiments to measure cholesterol, triglyceride, LDL, and HDL levels. All lipid fractions were measured using Humalyzer 3500 according to the kits' instructions.

### Statistical analysis

All data are presented as mean  $\pm$  SEM. Kolmogorov-Smirnov analysis is used to test data distribution, while Levine's test is used to determine the data's homogeneity. Normally distributed data were analyzed using one-way ANOVA followed by a Least Significance Difference (LSD) post hoc test. Meanwhile, not normally distributed data were analyzed with a Kruskal-Wallis analysis, followed by a Mann-Whitney test. A significant difference was considered achieved when  $P < 0.05$ .

## RESULTS and DISCUSSION

Dyslipidemia in diabetic patients is common since insulin-dependent pathways of lipid metabolisms were considerably altered<sup>12</sup>. Effective treatment of diabetic-induced dyslipidemia can significantly reduce the risk of cardiovascular disorders. In an effort to find an effective treatment for diabetic-induced dyslipidemia, this study examined the effectiveness of *Artocarpus altilis* leaf extract in improving lipid metabolism in diabetic rats.

In this study, 25 out of 45 rats experienced hyperglycemia or increased blood glucose level after 72 hours from alloxan (155 mg/kg BW) injection. A previous review has pointed out that alloxan's diabetogenic effect could be unpredictable, as alloxan may induce diabetes in 33% to 60% of rats injected with 150 mg/kg to 170 mg/kg of alloxan<sup>13</sup>. Some factors may influence alloxan diabetogenic effects, including the route and rate of injection, animal age, and type of diet<sup>14</sup>. Two different pathogenesis were associated with alloxan-induced diabetes: 1) through a selective inhibition of insulin secretion by specific inhibition on glucokinase; 2) induction of the formation of ROS, causing selective necrosis of beta-pancreatic cells.

The increase in blood glucose levels varied among rats after alloxan injection, but most rat BG levels reached  $>300$  mg/dl, and the average BG levels were not significantly different among groups. Figure 1 shows the blood glucose level after 14 days of treatment administration. It is found that only the highest dose of *Artocarpus altilis* extract (400 mg/kg) significantly reduced the blood glucose level, which was similar to that seen with insulin treatment ( $p < 0.05$ ).

The body weights of alloxan-induced diabetic rats are depicted in Table 1. Eighty percent of the placebo group, while only 40% of rats treated with extract 400 mg/kg or insulin, experienced weight loss.

**Table 1.** The profile of rat body weight before alloxan injection (baseline), after treatment, and overall changes in body weight of rats

Groups	Baseline weight (a)	Post-treatment (b)	Weight change (b-a)	Percentage of an animal with weight loss (%)
Normal (n=5)	243.4±20.0 g	259.2±18.3 g	+15.8±6.0 g	0
Placebo (n=5)	210.4±15.3 g	188.8±13.3 g	-21.6±13.1 g	80
Ext 100 (n=5)	239.0±17.8 g	224.6±35.5 g	-14.4±19.4 g	40
Ext 200 (n=5)	232.4±17.8 g	216.0±17.8 g	-16.4±9.3 g	80
Ext 400 (n=5)	206.0±3.3 g	205.0±16.2 g	-1.0±14.3 g	40
Insulin (n=5)	226.0±14.4 g	225.2±7.3 g	-0.8±11.3 g	40

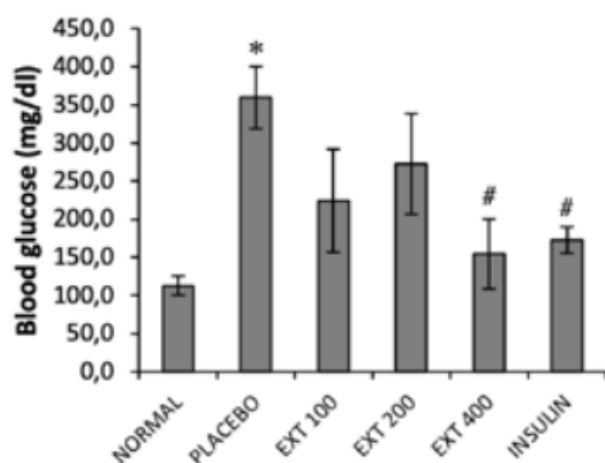
+ weight change=weight gain; -weight change=weight loss

Weight loss could happen as the body has to compensate for the lack of energy production from glucose by switching the source of ATP to non-carbohydrate molecules, such as fat and protein from muscle tissues<sup>15</sup>. The body weight changes varied in each treatment group. While the normal group gained weight after 14 days of the experiment (15.8±6.0 g), a significant weight loss was shown in the placebo, extract 100 and extract 200 groups, with an average of weight loss of 21.6±13.1 grams, 14.4±19.4 grams, and 16.4±9.3 grams, respectively. Meanwhile, the insulin group and the 400 mg/kg *Artocarpus altilis* extract group did not experience a marked decrease in their body weights. This result could suggest a potential role of *Artocarpus altilis* leaf extract to improve glucose metabolism in diabetic rats.

Figure 2 shows the levels of total cholesterol, triglycerides, LDL, and HDL in alloxan-induced diabetic rats following 14 days of treatment. It is revealed that the normal groups had total cholesterol (TC) levels of 41 to 116 mg/dl, with an average of 71 ±12.3 mg/dl. Meanwhile, the diabetic rats that only received a placebo had increased TC level (175 ±22.8 mg/dl), which was significantly elevated compared to normal control. The *Artocarpus altilis* extract of 100 mg/kg, 200 mg/kg, and 400 mg/kg were found effective to lower the TC levels of alloxan-induced diabetic rats ( $P<0.05$ ), which was similar to that achieved with the insulin treatment ( $P<0.05$ ).

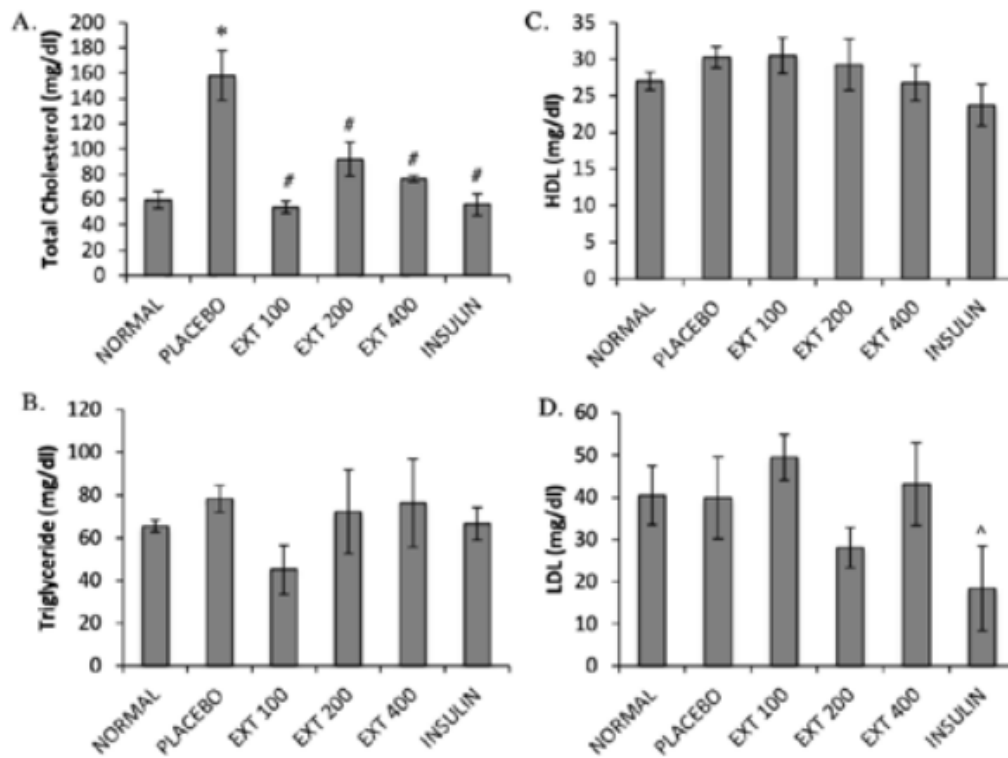
As seen in Figure 1, the placebo group had a slightly increased triglyceride level compared to the normal group (65.6±2.7 mg/dl vs. 78.3±6.3 mg/dl); however, it did not reach statistical significance. Out of the treatments given, only 100 mg/kg of *Artocarpus altilis* extract administration resulted in a reduction of

triglyceride level compared to the placebo group. Unlike the TC and triglyceride levels, the HDL levels of diabetic rats were not significantly altered compared to the normal group. Interestingly, there was an increase in LDL level with 100 mg/kg of *Artocarpus altilis* extract and a reduced LDL level with insulin treatment; yet, these changes did not reach statistical significance compared to the normal group.



**Figure 1.** The blood glucose levels in non-diabetic (normal) and diabetic rats after receiving a placebo, *Artocarpus* leaf extract 100, 200, 400 mg/kg, or insulin injection. \* $P < 0.05$  between the placebo group and normal controls. # $P < 0.05$  between treatment groups and placebo.

The common characteristics of diabetic dyslipidemia include hypertriglyceridemia, low HDL cholesterol, and elevated LDL, with hypertriglyceridemia being more dominant<sup>16</sup>. Interestingly, alloxan injection in this study only slightly induced hypertriglyceridemia in rats. The discrepancy may originate from the fact that alloxan-induced diabetes had different mechanisms from that develop in humans.



**Figure 2.** The lipid profiles of non-diabetic (normal) and diabetic rats after receiving placebo, Artocarpus leaf extract 100 mg/kg, 200 mg/kg, 400 mg/kg, or insulin injection. A. Total cholesterol; B. Triglyceride; C. High-density lipoprotein; D. Blood glucose. \* $P < 0.05$  between the placebo group and normal controls. # $P < 0.05$  between the treatment and placebo groups. ^ $P < 0.05$  between the insulin and EXT 100 groups.

Alloxan shares a similar molecular structure with glucose, hence, its cellular uptake is also facilitated by GLUT2 in beta-pancreatic cells. Alloxan diabetogenicity comes from the selective inhibition of glucose-stimulated insulin release through glucokinase inactivation and ROS production<sup>17</sup>. Meanwhile, the development of diabetes mellitus in humans is far more complex, involving a range of factors, including genetics, nutritional state, and environment<sup>18</sup>. Dyslipidemia found in diabetes mellitus type 2 patients is progressively developed due to insulin resistance. In contrast, alloxan injection acutely damages the Langerhans islands of the pancreatic tissues, leading to a degeneration of beta-pancreatic cells and a massive reduction in insulin production and release<sup>19</sup>. This form of diabetes is repeatedly associated with persistent ketoacidosis and hypercholesterolemia<sup>20</sup>; yet, a significant increase in LDL and triglyceride levels or a reduction in HDL levels may inconsistently be observed in alloxan-induced diabetic rats<sup>21, 22</sup>.

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At the dose of 400 mg/kg, *Artocarpus altilis* extract improved blood glucose levels and prevented weight loss in alloxan-induced diabetic rats. In addition to its hypoglycaemic effect, the administration of *Artocarpus altilis* leaf ethanol extract significantly improved total cholesterol level compared to placebo ( $p < 0.05$ ) and triglycerides, to a lesser extent, with the low dose. The effect of *Artocarpus altilis* leaf extract treatments was similar to insulin in reducing total cholesterol levels in alloxan-induced diabetic rats. This result may indicate the potential roles of *Artocarpus altilis* leaf extract as an alternative treatment for diabetic hypercholesterolemia. Another study also reported the antidiabetic effect of *Artocarpus altilis* extract occurred along with an improvement in histological structures of the islets of Langerhans<sup>23</sup>. Indeed, the protective effect of *Artocarpus altilis* extract has also been demonstrated in the liver and kidneys of alloxan-treated animals<sup>24</sup>. The antidiabetic, antihypercholesterolemic, and organ protective effects of the *Artocarpus altilis* leaf extract may be related to the presence of high content of a range of phytochemicals content, including polyphenol compounds and flavonoids. It has been shown that flavonoids and flavonoid-rich extracts, such as luteolin, curcumin, wild berry extract, hawk tea, and anthocyanin extract, may inhibit cholesterol uptake via inactivation of a cholesterol transporter, the Niemann–Pick C1-like 1 (NPC1L1) protein, resulting in a decrease of blood cholesterol levels<sup>25</sup>. Further study is still warranted to elucidate the specific bioactive compounds and the putative mechanisms underlying the therapeutic potential of *Artocarpus altilis* extract.

#### STATEMENT OF ETHICS

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All animal procedures have been approved by the institutional ethics committee at the Faculty of Medicine, Hasanuddin University, with an ethical clearance number of 544/UN4.6.4.5.31/PP36/2019.

#### 6 CONFLICT OF INTEREST

The authors declare to have no conflict of interest

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