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Antidiabetic Activity of Papaya Leaf Extract (*Carica Papaya* L.) Isolated with Maceration Method in Alloxan-Induces Diabetic Mice

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder disease that is often found in the world. Treatment with natural products can be an effective alternative in treating diabetes mellitus. Papaya (*Carica papaya*) has many pharmacological activities that have potential as an antidiabetic. **Objective:** This study aimed to determine the content of compounds in the papaya leaf ethanol extract and the effect of papaya leaf ethanol extract (*Carica papaya* L.) on the blood sugar level of diabetic mice induced by alloxan.

Methods: The mice were adapted for 7 days and were induced on the 8 days with 180 mg/kg alloxan intraperitoneally. Papaya leaf ethanol extract (*Carica papaya* L.) was given for 14 days at a dose of 250, 500, and 1000 mg/kg body weight and was compared to distilled water and alloxan only as negative control and glibenclamide at a dose of 2 mg/kg body weight as the positive control. Blood samples were collected on day 1, 7, and 14 to evaluate blood glucose levels before and after treatment.

Results: The compounds contained in the ethanol extract of papaya leaf include alkaloids, flavonoids, triterpenoids, tannins, and saponins. The administration of papaya leaf ethanol extract with 3 doses and glibenclamide at a dose of 2 mg/kg body weight could reduce blood glucose levels in diabetic Wistar mice induced by alloxan. The administration at a dose of 1000 mg/kg body weight of papaya leaf ethanol extract is more effective in reducing blood glucose levels in diabetic Wistar mice compared to 2 mg/kg body weight of glibenclamide.

Conclusion: Induction of *Carica papaya* leaf extracts decreased blood sugar levels but cannot effectively increased the body weight in diabetic mice.

Keywords: Antidiabetic, Ethanol Extract, *Carica papaya* L., Wistar Mice, Alloxan

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INTRODUCTION

Diabetes is a metabolism disorder that found in human and animal. The most frequent in diabetes is type 2 diabetes (1,2) , whereas this disorder tends to increase by the year. Moreover, one of the most important factors in diabetes treatment is controlling in blood glucose levels with close attention to the nutrients consumed, including the type of food that cause increased blood sugar (3,4). Organisms with diabetes mellitus are usually treated with modern medicine, which is consisted of oral hypoglycemic medicine, insulin injection, and other antidiabetic injections (5). Glibenclamide is a medicine from sulfonylurea group that is often used to type 2 diabetes treatment (6,7). However, this medicine has several adverse effects (8,9). Side effects including hypoglycemia, nausea, and skin reactions such as erythema multiforme, exfoliative . Occasionally, they can cause abnormalities in liver function tests, which may rarely lead to cholestatic jaundice, hepatitis and hepatic failure (6,31). Using glibenclamide frequently can cause fatal hypoglycemia and liver abnormalities. Therefore, the treatment with alternative medicine become an innovation in the antidiabetic medicines development with no side effects.

Based on the abundant natural product potential in Indonesia, exploration is needed to find alternative medicine as antidiabetic (10,11). One of the plants that can be utilized as an antidiabetic medicine is papaya (*Carica papaya*) with its leaf as an antidiabetic agent. Several previous studies reported that *Carica papaya* leaf has the potential to become an antidiabetic medicine. Juárez-Rojop et al. (2014) revealed that the *Carica papaya* leaf had a hypoglycemic effect on mice induced with streptozotocin (STZ) (12). The results of Airaodion et al. (2019) extract of *Carica papaya* leaf had a hypoglycemic effect on mice induced with alloxan (13). Based on this previous result, this study attempt to innovate with the use of ethanol solvent and maceration method to obtain *Carica papaya* leaf extract. Moreover, the *Carica papaya* leaf extract in this study was extracted using the maceration for application on a large scale and easy to use by the community. Therefore, this study investigated *Carica papaya* leaf extract obtained through the maceration method on the hypoglycemic effect in mice induced with alloxan.

MATERIALS AND METHODS

Animals

The animals used in this study were Wistar mice weighing between 25-35 g. The mice were acclimation for 7 days. They were placed into the cage, given clean water *ad libitum* and standard commercial pellet feed. Ethical code, institutional and national regulation on live animals were strictly observed. Animals used in this study received ethical approval from Health Research Ethical Clearance Commission Faculty of Dental Medicine

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Preparation of *Carica papaya* Leaf Extract

The leaves were rinsed with distilled water, then dried with oven at 60°C and grinded into a crude material with around 1 mm of diameter. Extraction was performed with cold extraction using the maceration method into 96% ethanol solvent for 48 hours using the "intermittent shaking" method to obtain an extract. The extract was evaporated by means of a rotary evaporator at 50 rpm at 40°C until a concentrated extract was obtained. The concentrated extract was then placed in a beaker glass and closed with an aluminum foil and stored in a refrigerator at 4°C to avoid damage. The solvent used was Sodium carboxylmethyl cellulose (CMC Na) with 1% concentration to obtain papaya leaf extract with several concentrations.

Flavonoids Test

A sample of 1 g was added to 10 mL of hot water, boiled for 5 min, and filtered while hot. Then 5 mL was taken and added with 0.1 g of Mg powder, 1 mL of concentrated hydrochloric acid and 2 mL of amyl alcohol, shaken and left to separate. The color produced in the amyl alcohol layer was observed.

Tannins Test

A sample of 5 g was mixed with 10 mL of distilled water, filtered, then the filtrate was diluted with distilled water until it was colorless. Then 2 mL of the solution are added to 1 to 2 drops of ferric chloride reagent.

Alkaloids Test

A sample of 0.5 g was added with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water, heated on a water bath for 2 min, then cooled and filtered. The filtrate is used for the alkaloids test. 3 test tubes were taken and then 0.5 mL of the filtrate was inserted into each test tube. In each tube 2 drops of reagent were added, and the results were observed.

Steroids / Triterpenoid Test

A sample of 0.5 g was dissolved with ethanol with ether added in a porcelain dish, then evaporated to dryness. Then, add 5 drops of concentrated H_2SO_4 and 3 drops of anhydrous acetic acid.

Saponins Test

A sample of 0.5 g was mixed with 10 mL of hot water, then cooled and shaken vigorously for 10 s to get foam. Then add 1 drop of 2 N HCl to observe the foam resistance, the presence of a steady foam indicates a saponin.

Acute Toxicity Test

A sample of 20 Wistar mice were randomly divided into 4 treatment groups (A-D), consisting of 5 mice per group. They were given papaya leaf ethanol extract (*Carica papaya* L.) with 100, 500, 1000, and 3000 mg/kg oral dose using a gastric gavage. The mice were given clean feed and drink ad libitum, then observed for 14 days to determine toxicity and mortality.

Subacute Toxicity Test

A sample of 20 Wistar mice were randomly divided into 4 treatment groups (A-D) consisting of 5 mice per group. They were given papaya leaf extract (*Carica papaya* L.) with 100, 500, 1000, and 3000 mg/kg dose orally every day for 4 weeks. The mice were given feed and drink ad libitum, then observed every day to determine toxicity and mortality.

Antidiabetic Test

This test was conducted using the method described by Ibeh and Ezeaja (2011) with a few modifications (14). Wistar mice were induced with alloxan monohydrate injection dissolved in 0.9% saline buffer solution with 180 mg/kg body weight dose, given intraperitoneally. The fasting blood glucose levels of each mice was measured on day 5 after alloxan injection using a glucose kit. After 8 days, animals with fasting blood glucose of 250 mg/dL or more were considered diabetic and were used for the study. The animals were divided into five groups, consisting of five mice in each group as followed: Normal control group with only distilled water, positive control group was given glibenclamide on 2 mg/kg body weight dose, model control group was given alloxan only with 180 mg/kg body weight dose, *Carica papaya* treatment groups were given *Carica papaya* leaf extract, each group of 250, 500, and 1000 mg/kg body weight dose, respectively. All of treatments were given orally with a gastric gavage. The fasting blood glucose levels of the mice was measured on day 1, 7, and 14 after treatment. Blood samples were collected and blood glucose was measured with Accuchek instant glucometer kit. This instrument has several advantages, including the small sample size needed (0.6 µL), easy to use, and the result is fast, only within 4 s.

Data Analysis

All research data were analyzed using the SPPS 22 software. One-Way ANOVA test was used to determine the significance. Differences between groups were tested using the Tukey test. A significant difference was found at $P < 0.05$.

RESULTS

Acute Toxicity Test

During the 7 days of the test, there was no mortality in mice after oral administration of a single dose (100, 500, 1000, 3000 mg/kg body weight) from *Carica papaya* leaf extracts, there was no evidence of toxicity or death was recorded. This result showed that lethal dose 50 (LD_{50}) value from the Carica papaya leaf extracts was higher than 3000 mg/kg body weight.

Subacute Toxicity Test

After regular daily administration of ethanol extract leaf from *Carica papaya* L. for 4 weeks, no evidence of toxicity or death was recorded and there were no significant changes in body weight at the end of treatment with *Carica papaya* L. leaf.

Phytochemical Screening of *Carica papaya* Leaf Extracts

This result showed that the *Carica papaya* leaf extract contained alkaloids, flavonoids, triterpenoids, tannins and saponins as shown in Table 1.

Table 1. Phytochemistry of the ethanolic leaf extracts of *Carica papaya*

Phytochemical test	Ethanol extract
Flavonoids	+
Tannins	+
Saponins	+
Triterpenoids	+
Alkaloids	+

Note: (+) showed a positive result from *Carica papaya* leaf extracts

Antidiabetic effect of *Carica papaya* leaf extract on blood glucose levels

The results of this study indicated that alloxan administration increased blood glucose levels significantly in comparison with the normal control group ($P < 0.05$). Induction of *Carica papaya* leaf extract and glibenclamide reduced blood glucose levels significantly compared to the model control group ($P < 0.05$). In addition, continuous induction of *Carica papaya* leaf extracts decreased blood glucose levels to resemble the normal group value, especially at a dose of 1000 mg/kg body weight (Table 2).

Table 2. Effects of *Carica papaya* leaf extracts on blood glucose levels in mice after 14 days treatment.

Groups	Blood Glucose Levels (mg/dL)		
	Day 1	**Day 7**	**Day 14**
Normal	112.4±1.95	115.4±1,82	112.6±3.21
Glibenclamide	354±3.39**	243.6±12.22**##	105±3.16**##
Model	324.8±4.82**	392.8±4.82**	398.8±4.82**
Carica Papaya leaf extract 250	308.2±3.35**##	267±5.87**##	183.6±3.78**##
Carica Papaya leaf extract 500	325.4±4.62**	240.4±13.3**##	170.6±4.67**##
Carica Papaya leaf extract 1000	334±3.74**#	164.4±17.73**##	110.2±5.63##

Data express the mean ± SD ($n = 5$). $^{*}P < 0.05$ compared between normal control group and treatment groups. $^{**}P < 0.01$ compared between normal control group and treatment groups. $^{\#}P < 0.05$ compared between model control group and treatment groups. $^{\#\#}P < 0.01$ compared between model control group and treatment groups.

Antidiabetic effect of *Carica papaya* leaf extract on body weight

Alloxan administration did not significantly reduce body weight of mice ($P > 0.05$). Meanwhile, the induction of *Carica papaya* leaf extracts and glibenclamide did not significantly increased the body weight of mice ($P > 0.05$). Induction of *Carica papaya* leaf extracts consecutively for 14 days was not able to increase body weight effectively beyond the model control group.

Table 3. Changes in the mean body weight of mice after 14th days treatment with *Carica papaya* L. leaf ethanolic extract.

Groups	Body Weight (g)		
	Day 1	**Day 7**	**Day 14**
Normal	32.76±1.62	32.69±1.7	32.69±1.73
Glibenclamide	29.93±2.7	29.02±2.67*	29.91±2.82
Model	33.06±1.62	30.83±1.92	28.78±1.55
Carica Papaya leaf extract 250	26.66±1.55**##	28.44±0.96*	28.78±2.67
Carica Papaya leaf extract 500	30.62±3.27	29.62±2.91	30.46±4.9
Carica Papaya leaf extract 1000	27.69±0.44**##	26.98±1.18**	26.7±0.87

Data express the mean ± SD ($n = 5$). $^{*}P < 0.05$ compared between normal control group and treatment groups. $^{**}P < 0.01$ compared between normal control group and treatment groups. $^{\#}P < 0.05$ compared between model control group and treatment groups. $^{\#\#}P < 0.01$ compared between model control group and treatment groups.

DISCUSSION

A toxicity test was conducted to determine the safety of extracts in body metabolism. There are two toxicity tests such as acute and subacute. An acute toxicity test is important to measure and evaluate the toxic characteristics of a chemical substance. This test provides information on the danger to human health derived from chemical substances exposed orally to the body in the short-term. Meanwhile, subacute toxicity tests are conducted to determine the long-term effects of the drug. The parameters that are often used are the presence of toxicity and mortality in mice. This research was performed to validate scientifically usage of *Carica papaya* leaf in ethno-medicine used in treating *in vivo* and *in vitro* diabetes mellitus. Oral administration of *Carica papaya* leaf extracts were well tolerated in all doses (100 – 3000 mg/kg body weight) with no mortality was observed in 4 weeks. The animals displayed no visible clinical signs, such as somnolence, depression, decreased movement and clumping together.

There was no toxicity in *Carica papaya* leaf extracts even at the dose of 3000 mg/kg body weight. This is confirmed by the findings of Fabricant & Farnsworths (2001) which stated that medicinal plants have been described as having an advantage in the estimation of toxicity based on their long-term usage by humans and as such the bioactive compounds derived from these plants could have low toxicity in animals and people (15,16). Mice were induced with alloxan to increase their blood glucose levels. The blood glucose levels of the mice was measured

on day 5 after alloxan injection with a glucose kit. After 8 days, mice with ≥ 250 mg/dL of fasting blood glucose were considered diabetes and were used for study. Alloxan induced to the peritoneum of experimental animal can cause selective damage to pancreatic beta cells (17). Alloxan is an agent that causes diabetes mellitus (18). In vitro, alloxan causes pancreatic beta cells to undergo necrosis by stimulating intracellular H_2O_2 (19,20). Alloxan causes hyperglycemia within 2-3 days. It also inhibits cell hemostasis, which is early cell death due to the disturbance of the cell oxidation process. An increased concentration of calcium ions accelerates the damage to pancreatic beta cells. This damage leads to disturbance in insulin secretion, which decreased the number of insulins (21). This causes the incapability form from the body to use glucose as a source of energy (22).

The treatment was given on day 8, which was a day after increased blood glucose levels and it was given for 14 days. The results of blood glucose levels showed that the administration of glibenclamide and *Carica papaya* leaf extracts decreased blood glucose levels effectively. The ethanol extract of *Carica papaya* L. administered to diabetic mice was effective in reducing blood glucose levels after 14 days of the administration, compared to the model control group. On day 7 and day 14 after treatment, the positive control group and treatment group showed a gradual decrease in blood glucose level. The normal control group showed normal blood glucose of <126 mg/dL. This indicated that the pancreas still functions normally in regulating blood glucose levels. In normal conditions, glucose from food is transported through the portal vein by glucose transporter in the intestines. Meanwhile, the model control group still experienced hyperglycemia. The quickest reduction of blood glucose level was seen in the group that was given extract with 1000 mg/kg body weight dose, compared to other groups. The effect was increased along with the dosage increase of *Carica papaya* extract. Data analysis showed that the most effective dose for *Carica papaya* for antidiabetic was 1000 mg/kg body weight. The antidiabetic activity of papaya leaf ethanol extract occurred because of chemical substances contained within the leaf that worked synergically in reducing blood glucose levels. The mechanism of blood glucose reduction depends on the mechanism of each substance. Furthermore, the active substance contained in papaya leaf also acts in stimulating the release of insulin from beta-pancreatic cells and the release of somatostatin and suppress the secretion of glucagon (23).

Carica papaya leaf extract and glibenclamide have antidiabetic activities on mice induced by alloxan. Glibenclamide is an oral hypoglycemic antidiabetic drug derived from sulfonylurea which actively reduces blood glucose level (24). Glibenclamide works by stimulating insulin secretion from the granules of pancreatic Langerhans beta cells through interaction with the ATP-sensitive K channel in the beta cell membrane. This causes membrane depolarization, which will open the Ca channel. The opening of the Ca channel will lead Ca^{++} ions into beta cells, stimulating granules filled with insulin, which lead to insulin secretion (25). This study found that *Carica papaya* leaf ethanol extract also had several chemical substances, including saponins, flavonoids, terpenoids, tannins, and alkaloids, which causes hypoglycemia. Flavonoids, terpenoid, saponins, and tannins give antioxidant activities, which can capture free radicals produced by the oxidation reaction of alloxan and reduce oxidative stress (26). Alkaloids and saponins can stimulate the secretion of insulin from pancreatic beta cells (27,28). Terpenoids, like triterpenoids, can increase the absorption of glucose by copying the function of insulin and as an insulin sensitizer.

Alkaloids tends to release insulin from pancreatic beta cells and can protect pancreatic beta cells from damage due to alloxan induction on animal models. Saponins has an antioxidant activity that protects beta cells and reduces the degranulation of insulin. Saponins improve clinical symptoms of diabetes significantly, including high blood glucose levels, and it plays role in the mechanism of enzyme α glucosidase inhibitor. Flavonoids in the process of regeneration of pancreatic beta cells by against free radicals (26), increase insulin release, and stimulate the absorption of Ca^{2+} from cell tissues which is very effective in the condition of a lack of insulin. Tannins is known to inhibit the loss of glucose transport which produced insulin. It is also suspected to induce phosphorylation from the insulin receptor by forming glucose 4 transporter (GLUT-4). Steroid reduced blood glucose by affecting insulin work at the cellular levels, distal insulin receptor, and reduced glucose production in the liver. According to results, body weight in mice was no decreased significantly after induction of diabetes as compared to normal control group. It means that alloxan induction cannot affect directly in body weight. Extracts induction also did not increased body weight significantly. This condition presence attributable to the reduction of the extract 's lipid activity or indirectly due to the effect on different lipid control systems (29,30,31).

CONCLUSION

The compounds contained in the *Carica papaya* leaf extracts include alkaloids, flavonoids, triterpenoids, tannins, and saponins. The extracts had no toxicity effect even in dose of 3000 mg/kg body weight. The administration of *Carica papaya* leaf extracts and glibenclamide decreased blood glucose levels in diabetic Wistar mice induced by alloxan but giving no effect in body weight.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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