

## *Piper crocatum* leaves extract reduced fasting blood glucose and glycated albumin but has lower efficacy than metformin in diabetic rats

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**Introduction:** *Piper crocatum* leaves extract has been widely proven in reducing blood glucose levels in rats. However, there is still no study that compared its effectiveness to metformin. Therefore, this study aimed to evaluate the efficacy of *Piper crocatum* leaves extract compared to metformin in reducing fasting blood glucose and glycated albumin.

**Methods:** Experimental pretest-posttest groups study using 16 adult male Wistar rats, aged 2-3 months, and weighing 200-220 grams was conducted. Diabetes was induced by using Streptozotocin and Nicotinamide intraperitoneally. The control group was treated with metformin 9 mg/200gBB/day while the treatment group was treated by *Piper crocatum* leaves extract at 0.6 g/200gBB/day for 14 days. Venous blood sample was used to assess fasting blood glucose and glycated albumin.

**Results:** The mean of fasting blood glucose level decreased

significantly in the control group from 182.00±19.38 mg/dl to 117.38±12.51 mg/dl ( $p<0.001$ ) and the levels of GA was also decreased from 3.04±0.99 ng/ml to 1.38±0.63 ng/ml ( $p<0.001$ ). In the treatment group, the level of fasting blood glucose decreased from 179.50±24.39 mg/dl to 140.13±15.12 mg/dl ( $p<0.001$ ) and the levels of glycated albumin was also decreased from 4.01±1.12 ng/ml to 3.58±0.90 ng/ml ( $p=0.036$ ). There was no difference in the level of fasting blood glucose and GA between the two groups before treatment but both parameters were significantly lower compared to the treatment group ( $p=0.005$  and  $p<0.001$  respectively).

**Conclusion:** Administration of *piper crocatum* leaves extract reduced fasting blood glucose but has lower efficacy compared to metformin in diabetic Wistar rats.

**Keywords:** *Piper crocatum* leaves, metformin, fasting blood glucose, glycated albumin, DM

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### INTRODUCTION

Aging is defined as a progressive physiological deterioration which leads to the onset of degenerative diseases and death. There are many factors that cause aging process that can be grouped into internal factors and external factors. The internal factors including free radicals, hormone levels disturbance, glycosylation, methylation, apoptosis, immune alteration and genes. The main external factors are unhealthy lifestyles, unhealthy diets and habits, environmental pollution, stress and poverty.<sup>1</sup>

Diabetes mellitus is one of the main health problems worldwide due to its high prevalence and increasing trend. WHO predicts that there will be an increase of diabetes prevalence in Indonesia from 8.4 million in 2000 to 21.3 million in 2030.<sup>2</sup> In definition, DM is a group of metabolic alterations characterized by hyperglycemia due to abnormalities of insulin secretion, insulin action or both. Diagnosis of DM is established according to laboratory finding of random blood glucose at  $\geq 200$  mg/dl or fasting blood glucose at  $> 126$  mg/dl.<sup>3</sup> Metformin is a good

standard for DM. Metformin is a biguanid group of drug that can reduce blood sugar levels through several mechanisms such as reducing liver glucose production (gluconeogenesis), improving glucose uptake in peripheral tissues and decreasing glucose hepatic output by activating of adenosine kinase monophosphate (AMPK).<sup>4</sup>

Currently, the glycated albumin (GA) is considered to have more diagnostic value for diabetes. GA is an indicator of glycemic control which is not affected by hemoglobin level and reflects a shorter blood glucose status than HbA1C, which is 2-4 weeks earlier. As a relatively new parameter, GA has several advantages as it is more stable, more sensitive and accurate to represent blood glucose fluctuations which underlie its promising future as a marker of medium-term glycemic control in DM patients.<sup>5</sup>

*Piper crocatum* leaves has been known to contain flavonoids which considered as strong antioxidants. The antioxidant content of *Piper crocatum* leaves

has been widely proven to reduce blood sugar levels in laboratory animals.<sup>6</sup> This effect is related to antioxidant compounds found in its leaves. Phytochemical analysis conducted at the Analytical Laboratory of Udayana University qualitatively showed that *Piper crocatum* leaves extract used in this study contained alkaloids, flavonoids, triterpenoids, saponins and phenolics (tannin). Quantitatively, examinations conducted at the Agricultural Technology Laboratory of Udayana University showed phenol content of 1.85% GAE, tannin of 12.43% TAE, flavonoids of 6112.24 mg/100g, antioxidant levels of 5693.16 mg/L GAEAC, and IC50% of 77.83 ppm.

Several studies have shown that flavonoids not only act as antioxidants but can play a role in carbohydrate metabolism. Flavonoids stimulate insulin sensitivity by modulating glucose transporters, stimulating glucose uptake in peripheral tissues, inhibiting glucose absorption in the intestine and increasing activity against enzymes involved in carbohydrate metabolism.<sup>7</sup>

Therefore, this study aimed to compare the effect *Piper crocatum* leaves to metformin on fasting blood glucose and glycated albumin in diabetic rats.

## METHODS

This research was an experimental study using a pretest-posttest control group design.<sup>8</sup> This study was conducted in an Integrated Biomedical Laboratory Unit of the Faculty of Medicine, Udayana University, Denpasar. The sample of this study were male Wistar rats (*Rattus norvegicus*), aged between 2-3 months, and weighing 200-220 grams. The

sample size was calculated using the Pocock (2008) formula, with a minimum sample size per group at 8 rats/group. The variables measured were levels of glycated albumin and fasting blood glucose after 14 days of treatment.

The rats were adapted for 7 days before being treated. On the 8<sup>th</sup> day, all rats were fasted for 8 hours and treated with a single dose of 13 mg/200g BW streptozotocin injections and 46mg/200g BW Nicotinamide intraperitoneally. Rats were considered DM if fasting blood glucose levels reached > 135 mg/dL. Sixteen DM rats were examined for fasting blood glucose and glycated albumin as a pretest data. Then, they divided randomly into 2 groups. The control group was treated with 9 mg/200gBB/day metformin of for 14 days and the treatment group was treated with 0.6 g/200gBB/day *Piper crocatum* leaves extract.

After 14 days of treatment, all rats from each group were fasted for 8 hours and then anesthetized by using inhaled ether. Blood (1 ml) were drawn through the medial canthus sinus orbitalis to measure fasting blood glucose levels and glycated albumin. Fasting blood glucose was examined with the glucose oxidase method and glycated albumin (GA) was measured by using ELISA.

## RESULTS

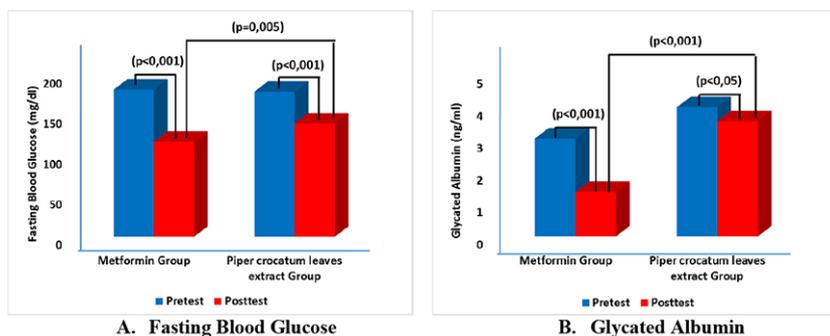
Prior to the treatment, there were no differences in fasting blood glucose and glycated albumin levels between the two groups ( $p > 0.05$ ). However, after 14 days of treatment, the mean fasting blood sugar level in the metformin group was lower ( $117.38 \pm 12.51$  mg/dl) compared to treatment group ( $140.13 \pm 15.12$

**Table 1. Comparison of Fasting Blood Glucose and Glycated Albumin**

Group	Pretest (Mean±SD)	Posttest (Mean±SD)	Mean Difference (Δ)	p*
<b>Fasting Blood Glucose (mg/dl)</b>				
Metformin Group	182.00±19.38	117.38±12.51	64.63±9.07	<0,001
<i>Piper crocatum</i> leaves extract Group	179.50±24.39	140.13±15.12	39.38±13.36	<0,001
<b>p**</b>	0.824	0.005		
<b>Glycated Albumin (ng/ml)</b>				
Metformin Group	3.04±0.99	1.38±0.63	1.66±0.46	<0,001
<i>Piper crocatum</i> leaves extract Group	4.01±1.12	3.58±0.90	0.43±0.47	0,036
<b>p**</b>	0.087	<0.001		

Ket: p\* = paired t test

p\*\* = independent t test



**Figure 1.** Graphical Comparison of Fasting Blood Glucose and Glycated Albumin

mg/dl;  $p=0.005$ ). In addition, the mean levels of glycated albumin in the metformin group was also lower ( $1.38\pm 0.63$  ng/ml) than *Piper crocatum* leaves extract group ( $3.58\pm 0.90$  ng/ml;  $p<0.001$ ).

Pretest-posttest analysis showed that there was  $64.63\pm 9.07$  mg/dl ( $p<0.001$ ) decrease in fasting blood glucose level while there was  $1.66\pm 0.46$  ng/ml decrease in glycated albumin level in the metformin group. Meanwhile, In the *Piper crocatum* leaves extract group, there was  $39.38\pm 13.36$  mg/dl ( $p<0.001$ ) decrease in fasting blood glucose levels while glycated albumin level decreased at  $0.43\pm 0.47$  ng/ml ( $p=0.036$ ).

## DISCUSSION

The results showed that *Piper crocatum* leaves extract was effective in reducing fasting blood sugar levels and glycated albumin. This effect is related to antioxidant compounds found in *Piper crocatum* leaves extract including tannins, flavonoids, and polyphenols. *Piper crocatum* leaves extract can reduce blood sugar levels through several mechanisms. The flavonoid content can stimulate insulin secretion in pancreatic beta cells and increase glucose uptake in cells and muscle tissue by stimulating Glucose transporters.<sup>7</sup> In addition, tannin can inhibit glucose absorption by forming layers in the intestinal mucosa.<sup>9</sup>

In addition, tannin content, flavonoids and polyphenols contained in *Piper crocatum* leaves extract reduced glycated albumin levels by inhibiting the glycation process. The presence of antioxidant content can inhibit the formation of amadori products in the reversible reaction phase of the millard reaction.<sup>10</sup> The increase in glycated albumin indicated an increase in the glycation process, so when the inhibition of glycation reaction occurs, the glycated albumin level will decrease.

This study also demonstrated that metformin was superior to *Piper crocatum* leaves extract in controlling glucose level. The main mechanism of metformin is by inhibiting liver glucose production (hepatic gluconeogenesis), slowing glucose absorption and increasing peripheral glucose uptake<sup>4</sup>. The molecular mechanism of metformin is to activate the AMP-activated Protein Kinase (AMPK) and stimulates fatty acid oxidation, glucose uptake, non-oxidative metabolism, and reduces lipogenesis as well as gluconeogenesis. The final effects are increased muscle glycogen content, decreased liver glucose production, increased insulin sensitivity, and lower blood glucose levels.<sup>11</sup> With more specific molecular mechanisms and specific active substances, metformin has a more potent mechanism of action in reducing fasting blood sugar levels and glycated albumin than those of *Piper crocatum* leaves extract.

## CONCLUSION

Based in the data, it can be concluded that the administration of *piper crocatum* leaves extract significantly reduced fasting blood glucose and glycated albumin in diabetic rats (*rattus norvegicus*) but not as effective as metformin.

## CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

## AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

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This study was self-funded without any contribution from third party.

## ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 405/KE-PH-Lit-2/VII/2019

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