The Role of Curcuma Zedoaria Extract on PlDF in Diabetic Nephropathy Wistar Rats

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Abstract. This study evaluated the effects of Curcuma zedoaria extract on blood glucose, Placental Growth Factor (PlGF), and kidney histopathology in diabetic nephropathy (DN) Wistar rats. DN, a severe diabetic complication, leads to progressive kidney damage. Using a post-test-only control group design, diabetes was induced in male Wistar rats with alloxan, and Curcuma zedoaria extract was administered orally at doses of 250 mg, 500 mg, and 750 mg for two weeks. Blood glucose, PlGF, and kidney histopathology were assessed. Data were analyzed using ANOVA, Kruskal-Wallis, and Bonferroni tests. Results showed significant blood glucose reduction ($p < 0.01$), with the 750 mg dose being most effective. However, no significant differences were observed in PlGF levels ($p = 0.902$) or kidney histopathology ($p = 0.152$). These findings suggest that high-dose Curcuma zedoaria effectively reduces blood glucose in DN, but its effects on PlGF and kidney histopathology are limited.

Keywords: Curcuma Zedoaria, Diabetic Nephropathy, PlGF, Blood Glucose, Kidney Histopathology

1 Introduction

Diabetic nephropathy is the most frequent complication of diabetes mellitus and an important cause of morbidity and mortality in diabetic patients. Oxidative stress, inflammation, and endothelial dysfunction are crucial components in the pathophysiology of DN. Diabetes affects 10.9% of adults in Indonesia, while 25% of these diabetic patients will develop DN [11]. This highlights DN as a critical health issue in Indonesia, with many patients at risk of severe renal decline without proper intervention.

The Curcuma Zedoaria, known locally as temu putih, is one of the plants traditionally used in medicine, where curcuminoids, flavonoids, and polyphenols have powerful anti-inflammatory and antioxidant properties. Such compounds exhibit anti-diabetic activity by enhancing $\beta - cellinsulinsecretion$, reducing glucose absorption, and increasing tissue glucose uptake [3, 19]. Additionally, increased Placental Growth Factor (PlGF) levels have been related to kidney injury in diabetes and may represent a biomarker for the monitoring of DN [20].

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This study aims to evaluate the effects of Curcuma Zedoaria extract on PlGF expression assess its protective potential against renal damage in a Wistar rat model of diabetic nephropathy and identify therapeutic agents that can inhibit or slow renal damage progression for improving patient quality of life. The motivation for this research lies in the need for safer, accessible natural therapies that mitigate the adverse effects of conventional treatments like leveraging the traditional use of Curcuma Zedoaria as an herbal remedy. Despite the known role of PlGF in exacerbating renal inflammation in DN, its modulation by Curcuma Zedoaria remains underexplored, which is why this study also tries to see if there's a relationship between Curcuma Zedoaria's effect on PlGF.

2 Literature Review

Diabetic nephropathy is a severe complication of diabetes mellitus; it is characterized by progressive renal dysfunction and is an important contributor to morbidity and mortality worldwide. While therapeutic strategies have improved, the prevalence of DN remains so high that alternative or complementary therapies are well warranted. This review collates existing literature on the potential of Curcuma Zedoaria, or white turmeric, as a therapeutic agent in DN and vascular growth factors such as Placental Growth Factor (PlGF) to illustrate how Curcuma Zedoaria might prove to be an efficient adjuvant or alternative therapy in DN, minimizing the use of conventional treatments with adverse effects.

2.1 Pathophysiology of Diabetic Nephropathy and its Correlations to PlGF

DN development encompasses a complex interaction of chronic hyperglycemia, oxidative stress, and inflammatory processes, which in turn enables endothelial dysfunction, glomerular basement membrane thickening, and mesangial expansion to ensure progressive renal injury [1,18]. PlGF is a pro-angiogenic member of the VEGF family that has been implicated in DN progression. Elevated levels of PlGF promote vascular permeability and angiogenesis, exacerbating glomerular injury and inflammation [5]. This positions PlGF as a promising therapeutic target in DN. PlGF predominantly expressed in pathological states, plays a critical role in angiogenesis and inflammation within the kidney. Elevated PlGF levels in DN are linked to proteinuria, endothelial dysfunction, and glomerulosclerosis, making it a marker for disease progression [20].

2.2 The Role of Curcuma Zedoaria in DN and its Impact on PlGF

Curcuma Zedoaria is an endemic flora used in Indonesian medicine. The ingredients include curcuminoids, flavonoids, and sesquiterpenes. All these compounds play an important role in mitigating the pathophysiological mechanisms of DN, such as curcuminoids, which act as an anti-inflammatory effect by inhibiting pro-inflammatory cytokines, including $TNF - \alpha$ and IL-6, through suppressing

the $NF\text{-}\kappa B$ signaling pathway and decreasing renal inflammation and fibrosis [9, 19]. Reduction of oxidative stress: curcumin can enhance endogenous antioxidant enzymes such as superoxide dismutase and reduce markers of oxidative damage [12]. Glucose regulation: extracts of Curcuma Zedoaria improve insulin secretion, enhance glucose uptake in tissues, and, therefore, help in regulating hyperglycemia-the primary driver of DN progression [3, 14].

2.3 Gaps End

Although the effect of curcumin on PlGF has been documented in other conditions, direct evidence for the modulation of PlGF by Curcuma Zedoaria in DN models is scant.

2.4 Conclusion and Future Directions

Curcuma Zedoaria is an exciting natural option for the treatment of DN, as it acts in multifarious ways: anti-inflammatory, antioxidant, and a modulator of glucose levels. PlGF, mostly pathological in expression, performs a critical role in angiogenesis and inflammation in the kidney. This then positions PlGF as a very promising therapeutic target in DN. However, the modulating role of PlGF and long-term clinical efficacy deserve further investigation; hence, this interests the author to research more about the effect of Curcuma Zedoaria in PlGF and its relation to DN.

3 Methods

This study aims to see the effect of the anti-hyperglycemic effect of Curcuma Zedoaria and its effect on PlGF and DN. These experiments use a post-test-only control group design. This experiment was conducted in the Laboratory of Pharmacy University of Sumatera Utara (USU). The design and protocol employed in this study have been reviewed and approved by the Research Ethics Committee of the Prima University of Indonesia (No. 080/KEPK/UNPRI/VIII/2024).

The Material used in this study consists of Curcuma Zedoaria, probiotic force, and alloxan. The animal used in this research is Rattus Norvegicus, it's populations were calculated using Federer's formula and resulted in 30 rats divided into six groups (normal control group (healthy rats without being induced to alloxan), negative control group (Diabetics rats without treatments), positive control group (Diabetics rats treated with probiotic), Extracts 250 mg (Diabetic Rats treated with 250 mg of Curcuma Zedoaria Extracts), Extracts 500 mg (Diabetic Rats treated with 500 mg of Curcuma Zedoaria Extracts), Extracts 750 mg (Diabetic Rats treated with 750 mg of Curcuma Zedoaria Extracts), with each group consists of six rats.

The Curcuma Zedoaria extracts begin with the Curcuma Zedoaria cleaned, peeled, and sliced thinly before drying at 50°C for 5 hours. The dried slices were then ground into powder. A total of 350 grams of the powder was macerated

in 2 liters of 95% ethanol for 2 days, followed by filtration and concentration using a rotary evaporator at 50°C to obtain a thick ethanol extract of Curcuma Zedoaria.

Alloxan was administered intraperitoneally with $130mg/KgBW$ dosage. After the hyperglycemia conditions were acquired on day 3 of the induction of the alloxan, all experiment subjects received intervention according to their group.

Fasting blood glucose levels were taken on day 1 before the induction of alloxan, Day 3 after the induction of alloxan, and weekly during the 2-week treatment period using a glucometer. On the 15th day, the experiment subjects were euthanized, and the sample of blood was collected for biochemical study of PlGF, and kidney organs were collected to examine the histopathology of the kidneys after the interventions.

Data were analyzed using SPSS (version 29). If the data were normally distributed and homogeneous, ANOVA was used followed by a post hoc test using the Bonferroni test. If the data violated normality assumptions, the Kruskal-Wallis test was used.

4 Results

Blood Glucose Level and PlGF were checked for normality and homogeneity. According to the Shapiro-Wilk test, the p-value was > 0.05 in all groups studied, which shows that they are normally distributed. In Levene's homogeneity test, the p-values were above 0.05, reflecting homogeneous variances; thus, it confirmed the adequacy of the parametric statistical test one-way ANOVA for further analysis, while histopathology was considered as nonparametric data so it would continue using the Kruskal Wallis test.

Table 1. Statistical Analysis of Blood Glucose Level, PlGF Level and Kidney Histopathology to Curcuma Zedoaria

Parameters	**Curcuma Zedoaria Group**	
	F	Sig
Blood Glucose Level	52.442	0.001
PlGF Level	0.306	0.902
Kidney Histopathology	8.070	0.152

The p-values for Blood Glucose Levels and PlGF were determined based on One-way ANOVA. Hence, Kidney Histopathology was conducted based on the Kruskal-Wallis Test. All the Curcuma Zedoaria groups had a significant difference in the Blood Glucose group ($p < 0.001$); no significant difference to the

PlGF group ($p < 0.902$); no significant difference to the Kidney Histopathology group ($p < 0.152$).

From Figure 1, it can be interpreted that all the experiment subjects excluding the normal control group, their blood glucose increase after the administration of alloxan indicated hyperglycemia, and after the administration of probiotics and Curcuma Zedoaria within 2 weeks, each group's blood glucose level appears to constantly decrease, with 750mg dose to have more significantly decreased than other groups. The reduction value of blood glucose level from the day of alloxan-induced, day 7, and day 14 (366 mg/dl, 254 mg/dl, and 135 mg/dl).

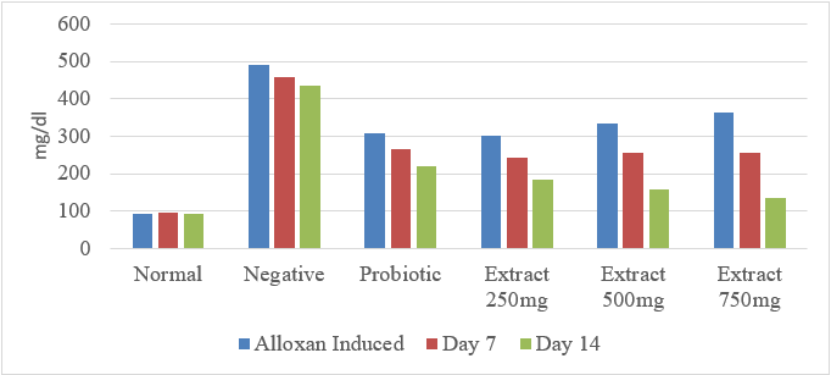

Fig. 1. Blood Glucose Level Change During the Experiment

Table 2. Post Hoc Analysis of Curcuma Zedoaria Relations to Blood Glucose Reduction using Bonferroni

Curcuma Extract 750mg		
Normal Group	56.97	< 0.001
Negative Group	51.10	< 0.001
Probiotic Group	32.33	< 0.001
Curcuma Extract 250mg	21.76	< 0.001
Curcuma Extract 500mg	9.48	< 0.001

From Table 2, the post-hoc analysis using Bonferroni showed that the highest mean reduction was in the 750 mg group against the normal control, with a mean difference of 56.97 and $p < 0.001$. In conclusion, Curcuma Zedoaria with 750mg

dose has the most effect in reducing blood glucose levels rather than negative group, probiotic group, Curcuma Extract 250 mg group and Curcuma Extract 500 mg group.

The normal control group displayed slightly higher mean levels of PlGF at 82.98 ± 17.18, while the lowest mean levels were in the probiotic control group at 74.13 ± 8.08; Figure 2. Since there is no significant variance, post-hoc analysis was not needed. This inconsistency in results may be due to the short, two-week duration of treatment, which might not have been long enough to significantly alter the expression of PlGF.

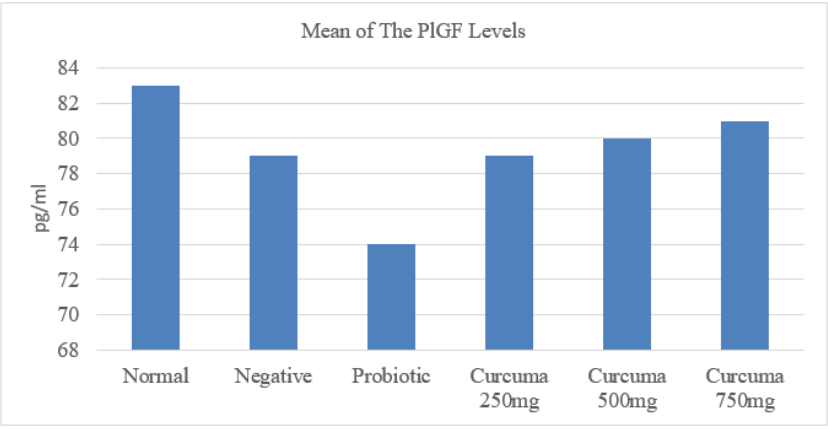

Fig. 2. Mean Distribution of PlGF levels in each Group

5 Discussion

This study aimed to determine the therapeutic potency of the Curcuma Zedoaria extract as an alternative treatment for diabetic nephropathy, emphasizing its role in the Placental Growth Factor and glycemic control. Diabetic nephropathy or DN is a common complication of diabetes. Chronic hyperglycemia, inflammation, and oxidative stress ultimately produce progressive structural and functional changes in the kidney. Curcuma Zedoaria exerts anti-hyperglycemic effects, anti-inflammatory and antioxidant properties that might potentially prevent the progression of DN.

Curcuma Zedoaria is a promising adjunct therapy in the management of DN. Its prominent anti-hyperglycemic feature brings into view its usefulness in metabolic control, a very important issue in the management of DN. Using natural antioxidants along with anti-inflammatory agents in the treatment may help to manage the multi-factorial pathogenesis of DN. Moreover, being a biomarker for PlGF will allow targeting for more accurate and personalized therapeutic interventions.

PlGF is a key biomarker mediator and important therapeutic in this process, associated with abnormal angiogenesis, vascular permeability, and the inflammatory cascades contributing to renal deterioration. These results add to the rapidly growing evidence of natural products as a complementary therapy in the management of DN, particularly in filling in the lacuna left by conventional treatments. The conclusion from this study is Curcuma Zedoaria effectively lowered blood glucose levels across the experimental groups, particularly at the 750 mg dose. However, the treatments did not yield significant level changes of PlGF or apparent improvements in kidney histopathology.

The results of the study showed that the administration of Curcuma Zedoaria extract to Wistar rats with diabetic nephropathy did not significantly change PlGF levels, and there was no significant difference in kidney histopathology between the treatment and control groups. This is in line with several other studies exploring the effects of Curcuma Zedoaria in the context of kidney disease and oxidative stress. For instance, a recent study by [6] showed that Curcuma Zedoaria might overcome oxidative damage, but the actual effects on specific biomarkers such as PlGF level were not discussed [6]. Furthermore, Handajani and Narissi reported that Curcuma Zedoaria oil can lower blood sugar levels in hyperglycemic rats, indicating that this extract has a therapeutic effect in the context of diabetes, although it does not directly affect kidney histopathology parameters [10].

Glycemic control observed is consistent with previous studies demonstrating Curcuma Zedoaria's ability to modulate metabolic pathways, including AMPK and GLUT4 activities [2,7]. Curcuma Zedoaria also shows some potential for antioxidant properties that may affect ROS. ROS are chemically reactive oxygen-containing molecules, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, that, in excess, can cause oxidative stress and subsequently damage cellular components.

Curcuma Zedoaria contains bioactive compounds such as curcuminoids, sesquiterpenes, and flavonoids, which have shown antioxidant activities. These components exert their antioxidant effects by either directly neutralizing ROS or by enhancing the endogenous antioxidant defenses of the body. Flavonoids in Curcuma Zedoaria can quench singlet oxygen or trap hydroxyl radicals, which neutralizes them and makes them less harmful molecules [16].

Curcuma Zedoaria can upregulate the expression and activity of endogenous antioxidant enzymes like Superoxide Dismutase (SOD), which catalyzes the dismutation of the superoxide anion into hydrogen peroxide and oxygen, Catalase (CAT), which breakdown hydrogen peroxide into water and oxygen, Glutathione Peroxidase (GPx), which can reduce hydrogen peroxide and lipid peroxides by using glutathione as a substrate [13].

Curcuma Zedoaria also can inhibit the ROS-mediated activation of $NF-\kappa B$, hence reducing inflammation and oxidative damage. And can inhibit the ROS-mediated activation of the MAPKs, which prevents apoptosis and cellular stress. Curcuma Zedoaria has been reported to decrease lipid peroxidation by

Scavenging ROS at lipid membranes and stabilizing cellular and mitochondrial membranes preventing ROS-induced damage [8].

This study has numerous limitations, which may affect its validity and generalizability. The short duration of the treatment did not allow the detection of signs of significant changes in chronic markers such as PlGF and kidney histopathology. Further, lacking phytochemical analysis, it is not possible to understand which active compounds can lead to such an effect. The choice of alloxan for the induction of diabetes may have underestimated the possible nephroprotective effects of Curcuma Zedoaria because alloxan is less effective than STZ in inducing the renal changes that are characteristic of DN.

Future researchers should target longer treatment periods to investigate long-term outcomes of Curcuma Zedoaria on PlGF and renal histopathology. Phytochemical characterization is needed to identify the responsible bioactive molecules for the therapeutic activity of the herb. Inducing diabetic models using STZ would be a more significant clinically relevant model for the assessment of nephroprotective activity rather than alloxan.

6 Conclusion

This study reinforces the potential of Curcuma Zedoaria as an alternative therapy for DN, particularly for glycemic control. This study revealed that Curcuma Zedoaria extract elicited a significant decrease in diabetic rats' blood glucose, with its highest dose of 750 mg of extract having the most intense action. Curcuma anti-hyperglycemic activities of curcuminoids and flavonoids mechanism by modulating vital metabolic pathways such as AMP-Activated Protein Kinase (AMPK) and Glucose Transporter 4 (GLUT4). The extract's efficacy in reducing ROS and hence oxidative stress, therefore, could be interpreted as one of the reasons for an improvement in pancreatic $\beta - cellfunction$ and insulin sensitivity.

Despite possessing anti-inflammatory and antioxidant activities. In this study, Curcuma Zedoaria extract did not significantly alter PlGF levels in the treated groups. PlGF is a critical marker of kidney injury in DN, which is involved in angiogenesis, vascular permeability, and inflammation. This inconsistency in results may be due to the short, two-week duration of treatment, which might not have been long enough to significantly alter the expression of PlGF.

In this study, histopathological analyses did not indicate any significant variation in renal morphology among treated versus control groups. Common markers of DN, such as glomerular hypertrophy, mesangial expansion, and tubular damage, did not change within two weeks. The short experimental period or using alloxan-induced diabetes, which causes less severe renal damage compared to STZ, can be a justification for such results.

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