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Potential Effect of *Orthosiphon aristatus* Leaf Extract in Improving Collagen 1 Alpha 1 Expression in Hyperglycemia-Induced Rats

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Abstract

Diabetes mellitus involves many mechanisms. The collagen metabolism pathway with very complex interactions and its regulation can contribute to the development of diabetes complications. The collagen 1 alpha 1 (COL1A1) plays an important role in the diabetes melitus pathway in adipose tissue by regulating adipocyte function, insulin resistance, and glucose metabolism and is identified as a potential therapeutic target for type 2 diabetes. The cat's whisker plant (*Ortosiphon aristatus*) pharmacologically has hypoglycemic activity and increases insulin expression. This study aimed to observe the potential of cat whisker leaf extract (*Ortosiphon aristatus*) in increasing the COL1A1 expression and is protective against increased blood sugar levels in hyperglycemia-induced *Rattus norvegicus*. The experimental design with pretest and posttest controlled group design was carried out by dividing 18 test animals into six groups: non-diabetic negative control, diabetic negative control, positive control (metformin), cat whisker leaf extract doses of 100, 200, and 400 mg/kgBW. Induction of hyperglycemia with streptozotocin 65 mg/kgBW and nicotinamide 230 mg/kgBW peritoneal injection. The extract treatment was given for 14 days. Blood sugar level measurements were carried out at the treatment's beginning and end. Adipose tissue was taken to examine COL1A1 expression by real time PCR technique by calculating the ratio of the cycle of tresh-hold COL1A1 to the internal gene β-actin. The results of Kruskall Wallis test showed no significant difference in COL1A1 expression between groups and the Spearman correlation test showed no relationship between blood sugar levels and COL1A1 expression ($p>0.05$). There was a decrease in blood sugar levels before and after treatment for all treatment groups.

Keywords: Diabetes Melitus; COL1A1 Expression; *Orthosiphon aristatus*; Real time PCR.

Abbreviations: COL1A1 = collagen 1 alpha 1, PCR = polymerase chain reaction

INTRODUCTION

Globally, deaths due to diabetes are projected to increase to 1.59 million and 79.3 million DALY (disability adjusted life years) by 20251, and increase to 7,079 people per 100,000 by 2030 with the burden of diabetes varying between regions. In Indonesia, the prevalence of diabetes mellitus is expected to increase significantly in the coming decades from 9.19% in 2020 (18.69 million cases) to 16.09% in 2045 (40.7 million cases), increasing by 75.1% over 25 years, or an average of 3% per year (Wahidin *et al*., 2024). The prevalence of type 2 diabetes follows a pattern that corresponds to socioeconomic development, with developed regions showing higher prevalence rates (Khan *et al*., 2019).

Diabetes mellitus is a metabolic disease characterized by disturbances in insulin secretion, insulin activity, or both. Insulin resistance (RI) in type 2 diabetes mellitus (T2D) is accompanied by dysfunction of glycolipid metabolism and protein biosynthesis. Over the past few decades, despite great advances in diabetes-related research, the discovery of specific molecular targets has not been enough. In addition, there is drug resistance and side effects related to the use of anti-diabetic drugs that can reduce the inhibition of insulin resistance. Risk factors for developing type 2 diabetes mellitus are obesity and insulin resistance (Lin *et al*., 2021).

The main characteristic of obesity and insulin resistance is the occurrence of extracellular matrix (ECM) remodeling abnormalities in muscle, liver, and adipose tissue. There is a growing body of evidence suggesting a link between ECM and insulin resistance. Although ECM remodeling is increasingly important in insulin resistance, effective ECM targets remain undefined. Collagen is the most abundant component of ECM, consisting of three α helical chains made of a series of repeating amino acids glycine-X-Y. An increase in collagen protein in insulin-resistant cells is associated

with increased mRNA levels. Increased expression of some of the genes that make up collagen proteins is associated with a high-fat diet. The increase in Col24α1 mRNA in the adipose tissue of obese white mice was consistent with the increase in other previously reported collagen isoform mRNAs including Col5α2, Col6α2, Col6α3 and Col18α1. Studies on the role of specific ECM components in insulin resistance are important but incomplete (Weng *et al*., 2020). COL1A1 is the most significant gene in the extracellular matrix receptor interaction pathway and is associated with hypoglycemic activity for the first time. Thus, Col1a1 is a potential new therapeutic target for reducing T2D (Lin *et al*., 2021).

Rapid advances in proteomics are critical to uncovering the mechanisms underlying T2D and identifying mechanism-based biomarkers. Tissue analysis correlated glycometabolic parameters and DEP (differentially expressed protein). The gene's expression level in the primary metabolic pathway is necessary to identify biomarker candidates. Collagen proteins interact with each other so further observation of collagen-encoding genes is needed so that a molecular network analysis related to insulin resistance is obtained (Lin *et al*., 2021; Weng *et al*., 2020). Diabetes mellitus as a chronic illness with considerable worldwide repercussions, necessitates appropriate management to avert fatal outcome. Natural pharmaceuticals are becoming increasingly popular due to their perceived safety compared to synthetic drugs, despite potentially poorer efficacy (Septiana *et al*., 2021).

Two hundred twenty-nine medicinal plants in Indonesia have been identified as beneficial for reducing blood glucose levels. The Asteraceae family and Orthosiphon aristatus species were the most predominant. It underscored the necessity confirming pharmacological activity at the molecular level (Arifah *et al.*, 2022). The leaves of the cat's whisker plant *(Ortosiphon aristatus*) contain various active substances (Nurcholis *et al*., 2022). The methanol and ethanol extracts of Ortosiphon aristatus exhibited remarkable α-glucosidase inhibitory activity, enhancing the recognition of the bioactive substances in Ortosiphon aristatus for diabetes management (Maulana *et al*., 2022). *Ortosiphon aristatus* extracts showed considerable hypoglycemics efficacy compared to the diabetes control group, less toxicity, suggesting promise for herbal treatment (Thinn *et al*., 2018).

The content of flavonoids plays a role in lowering blood sugar levels and modulating oxidative stress in the body by neutralizing the effects of nitrogen and oxygen species. Flavonoids help regulate carbohydrate digestion, insulin signaling, insulin secretion, glucose absorption, and adipose. Flavonoids target many molecules involved in regulating several pathways, such as increasing ß cell proliferation, increasing insulin secretion, reducing apoptosis, and improving hyperglycemia by regulating glucose metabolism in the liver (Ojo *et al*., 2023).

This study aimed to observe the potential of cat whisker leaf extract *(Ortosiphon aristatus)* in increasing the expression of the COL1A1 gene. Observation of the expression of the mRNA gene COL1A1 as a gene encoding one of the collagen proteins from adipose tissue is important because the COL1A1 gene is involved in the mechanism of insulin resistance with obesity risk factors. There is a contradiction between the results of the COL1A1 gene research with the protective properties of COL1A1 gene expression against increased blood sugar and vice versa.

MATERIALS AND METHODS

Study area

Extracts preparation, test animals treatment, blood sugar levels measurement, and adipose tissue collection were conducted at the Pharmacology Laboratory of the Faculty of Medicine, University of Muhamamdiyah Surakarta. Measurement of COL1A1 expression used the real-time PCR technique at the Integrated Research Laboratory of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta.

Procedures

Extract Preparation

The symplisia of cat whisker leaves (*Ortosiphon aristatus*) was obtained from B2P2TOOT (Center for Research and Development of Medicinal Plants and Traditional Medicine) Tawangmangu, Karanganyar, Central Java and the determination was obtained from the Tawangmangu Health Service UPF Laboratory. Symplisia is then put into a maceration pot then soaked using 70% ethanol, stirred for 1 hour per day for 5 days. The results of the soak are filtered with a filter cloth. Then it is evaporated with an evaporator to produce a viscous extract. The next step is the calculation of the dosage and the manufacture of the stock solution.

Animal preparation and diabetes induction

The experimental animal was a male *Rattus norvegicus* of the Wistar strain obtained from the Pharmacology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta. The inclusion criteria were healthy rats (normal activity), body weight 150-200 grams, 2-3 months old. The mice were fasted for 8 hours then divided into 6 groups (G) randomly and blood sugar measurements were taken (G1: non diabetic negative control, G2: diabetic negative control, G3: metformin, G4: *Ortosiphon aristatus* (cat whisker) extract dose 100mg/kgBW, G5: *Ortosiphon aristatus* extract dose 200mg/kgBW, and *Ortosiphon aristatus* extract dose 400mg/kgBW. Rats in the G2-G6 group were induced by hyperglycemic agent using nicotinamide (NTA) injection 230 mg/kgBB as pancreatic protector and streptozotocin (STZ) 65 mg/kgBB intraperitonially and were given standard feed and drinking water every day.

Blood sugar levels measurement

Blood sugar level measurements were carried out on day 0, day 4 after STZ and NTA induction, and day 14. Rats whose sugar levels had increased in the G2-G6 group were given cat whisker leaf extract treatment for 14 days orally. Following a 14-day period, blood glucose levels were evaluated, and the procedure was completed for the collection of adipose tissue.

Measurement of col1a1 expression

Approximately 30mg of rat adipose tissue was inserted into an RNA stabilizing solution for RNA extraction using phenol chloroform and cDNA synthesis according to the protocol Smobio Excel RT Kit II RP1400. COL1A1 gene expression was measured using real-time PCR according to the protocol of the SensiFAST Bioline Sybr Green Kit with a specific primer. Housekeeping gene β-actin was used as an internal control. COL1A1 expression was determined by measuring the cycle of tresh-hold (CT) ratio of the COL1A1 gene to the internal gene β-actin.

Table 1. Primer base sequence of COL1A1 and β-actin genes.

	Forward Primer	**Reverse Primer**
COL1A1	TGGCAAGAACGGAGATGA	AGCTGTTCCAGGCAATCC
β-actin	CTATCGGCAATGAGCGGTTCC	TGTGTTGGCATAGAGGTCTTTACG

Data analysis

The data on blood sugar levels and col1a1 expression obtained were statistically processed by the Kruskall Wallis non-paramteric test and the Spearman's rho correlation test.

RESULTS AND DISCUSSION

Based on blood sugar level data, there was an increase in blood sugar levels after induction of streptozotocin and nicotinamide in all groups (pre-test) and there was a decrease in blood sugar levels after administration of *Ortosiphon aristatu*s leaf extract (post-test). Because the pretest data is not homogeneous, the data on the difference in blood sugar levels of the pretest and the postest is used for analysis as presented in Table 1. As for the col1a1 expression data, there was no difference in expression between groups with a value of $p>0.05$.

Table 2. Result of blood glucose and col1a1 expression measurement.

Parameters		**Control (-) non diabetic**	**Control (-) diabetic**	**Control (+)**	**Group 1 (100mg/kgBW)**	**Group 2 (200mg/kgBW)**	**Group 3 (400mg/kgBW)**	**p value**
Blood glucose	Pre-posttest difference	21±19.13	15.33± 9.81	33.67±5.91	48.33± 20.37	35.67± 12.81	34± 30.41	>0.05*
Col1a1 expression	posttest	0,2±0,1	1,7±0,2	0,9±1,3	1,3±1,9	0,3±0,5	1,7±2,9	>0.05*
Blood glucose & Col1a1 expression								>0.05**

* : Kruskal Wallis test

** : Spearman's rho test

Discussion

This study showed that the administration of streptozotocin at 65 mg/kg body weight and NTA at 230 mg/kg body weight effectively elevated blood glucose levels by the 19th day post-induction, aligning with the mechanisms associated with Type 2 Diabetes (T2D). On the 19th day, fasting blood glucose levels were assessed, revealing an elevation relative to the baseline; this indicates successful induction of hyperglycemia in T2D. The pathogenesis of T2D involves several factors, including impaired insulin secretion by pancreatic ß cells and insulin resistance in peripheral tissues. This condition leads to a heterogeneous condition characterized by β cell failure, ultimately resulting in T2D. Streptozotocin has toxic properties because of its potential to damage cells β the pancreas and impact the expression of the GLUT-2 protein which causes increased blood glucose levels in test animals. STZ works by forming free radicals that damage pancreatic beta cells resulting in hyperglycemia and hypoinsulinemia (Munjiati *et al*., 2021).

Nicotinamide (NTA) effectively protects β cells against STZ cytotoxicity, NTA attenuates diabetes complications caused by streptozotocin and increases

survival rates in mice. Protection from try animals depends on decreasing the damage and methylation of deoxyribonucleic acid (DNA) in the pancreatic islets, increased insulin secretion, nerve protection function and. Nicotinamide (Pyridine-3-carbosamide) is an amide form of vitamin B3 or niacin. Nicotinamide is a percussor of Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADP) which can protect pancreatic β cells from various toxic substances. On the other hand, nicotinamide protects cells β the pancreas from DNA damage, therefore nicotinamide can cope with STZ-induced diabetes, increased hyperglycemia and weight loss (Cruz *et al*., 2019).

The differential test findings across groups indicated no significant differences, despite a reduction in blood glucose levels following the administration of Orthosiphon aristatus leaf extract. The extract's mechanism of action is intricate and not yet completely comprehended. STZ possesses the capability to limit DNA synthesis, induce cellular apoptosis, and hinder cellular regeneration (Harijanto & Dewajanti, 2017). The use of nicotinamide (NTA) is aimed at protecting against pancreatic damage, preventing it from becoming acute. Nicotinamide (NTA) effectively protects β cells against STZ cytotoxicity, NTA attenuates diabetes complications caused by streptozotocin and increases survival rates in mice. Protection from try animals depends on decreasing the damage and methylation of deoxyribonucleic acid (DNA) in the pancreatic islets, increased insulin secretion, nerve protection function and. Nicotinamide (Pyridine-3-carbosamide) is an amide form of vitamin B3 or niacin. Nicotinamide is a percussor of Nicotinamide Adenine Dinucleotide (NAD) and Nicotinamide Adenine Dinucleotide Phosphate (NADP) which can protect pancreatic β cells from various toxic substances (Cruz *et al.*, 2019).

This study observed the expression of col1a1 quantitatively using the analysis of the cycle of tresh hold (CT) value of the COL1A1 gene mRNA to the CT of the internal gene β-actin. The results of the COL1A1 gene expression difference test with the Kruskal Wallis test showed no significant difference in gene expression levels between groups, the same results were shown by the correlation test between blood sugar levels and gene expression with the Spearman's rho test with a value of $p>0.05$ (Table 2). This is possible because the expression process of the COL1A1 gene (col1a1 protein synthesis) is influenced by various factors both intracellular and extracellular, both at the transcription, post-transcription, and post-translational levels. The col1a1 protein is a protein that plays a role in the interaction of the extracellular matrix (ECM), the link between the col1a1 protein and diabetes mellitus occurs through the ECM-receptor interaction pathway (Lin *et al*., 2021).

The results of meaningless expression of COL1A1 genes between groups can be influenced by several aspects: (1) The synthesis and processing of COLA1 is a complex process involving intracellular modifications such as hydroxylation of proline, lysine, and glycosylation, which are important for the stability of collagen structure. Once secreted, procollagen undergoes proteolytic breakdown and the formation of cross-links in the extracellular matrix, which results in mature collagen fibrils. This process is controlled by various regulatory mechanisms, including epigenetics (DNA methylation, histone modification), transcription (activation by transcription factors such as Sp1, AP-1, and Runx2), post-transcription (regulation by miRNAs such as miR-29), as well as post-translational modification (hydroxylation, glycosylation, and proteolytic breakdown), which overall determine the final structure and function of collagen in tissues, (2) epigenetic regulation of COL1A1 occurs in some diseases, especially found at the transcription level which states that the TGFβ pathway plays an important role, especially through the regulation of the Smad transcription factor which interacts directly with COL1A1. Other factors known to play a role in the transcription process of COL1A1 are NF-κB, AP2, and NR4A1. Meanwhile, the involvement of other transcription factors such as NOTCH2, c-Myb, HIF1α, β-catenin, ZEB1, and YAP is still indirect, and further research is needed to ascertain whether these factors specifically target COL1A1, (3) there is COL1A1 regulation at the post-transcription level, namely the regulation of COL1A1 mRNA by the TENT5A gene and a number of miRNAs (miR-98, miR-126-5p, miR-218-5p, miR-328-3p, miR-338-3p and miR-29b-3p), (4) changes in post-translational modification of type I collagen or the occurrence of mutations that alter the pro-collagen proteolytic cleavage point, and (5) there is an influence of several proteins such as MMP-2 and tryptase-β that have implications for the regulation of COLA1(I) gene expression reported to be associated with various diseases (Devos *et al*., 2023).

Further research is needed to understand the effect of *Ortosiphon aristatus* on overall glucose metabolism. Several studies have shown the potential of *Ortosiphon aristatus* in lowering blood glucose levels, but the exact mechanism is still unclear. The results of the statistical test that did not differ significantly also assumed that the development of diabetes mellitus was not entirely in the chronic phase, related to the complex and long-term pathomechanism of diabetes mellitus.

CONCLUSIONS

Administration of cat whisker leaf extract (*Ortosiphon aristatus*) for 14 days was not significant in increasing the col1a1 expression of hyperglycemia-induced rats. Regarding the expression of col1a1, more comprehensive research is needed on the molecular processes involved in the synthesis of col1a1, by integrating various factors

that are currently still being carried out separately to clarify the potential of COL1A1-based therapies based on a thorough understanding of COL1A1 and COLα1 (I) regulation.

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Competing Interests: The authors declare that there are no competing interests.

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