The Effect of Andalas *(Morus macroura Miq.)* Tree Bark Extract on Il-1β Gene Expression in the Pancreas of Hyperglycemic Rats

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Abstract

Hyperglycemia, a defining feature of diabetes mellitus (DM), increases the production of cytokines that promote inflammation, including tumor necrosis factor alpha and interleukin-1 beta (IL-1β), which are regulated by the transcription factor NF-κB. This leads to chronic inflammation. While antidiabetic drugs are effective, their adverse effects warrant exploration of alternative therapies. This study evaluated the impact of Andalas *(Morus macroura Miq.)* tree bark extract on IL-1β gene expression in the pancreas of hyperglycemic rats. Six groups of 24 rats were used in the experiment: normal control (K−), hyperglycemic control (K+), metformin-treated (K), and three treatment groups where *Morus macroura* Miq. was administered at 100, 200, and 300 mg/kg of body weight (P1, P2, P3) tree bark extract. IL-1β gene expression was quantified, and blood glucose and body weight were monitored. The groups showed significant differences ($p = 0.032$) in mean IL-1β expression levels, which were K− (0.09), K+ (1.24), K (0.38), P1 (0.12), P2 (1.12), and P3 (1.76). The 200 mg/kg of body weight dose (P2) considerably lowered blood glucose and raised body weight, whereas the 100 mg/kg body weight dose (P1) most successfully suppressed IL-1β expression. These findings suggested that *Morus macroura* Miq. tree bark extract, particularly at 100–200 mg/kg of body weight, may offer therapeutic potential in mitigating hyperglycemia-induced inflammation.

Keywords*: Andalas (Morus macroura Miq.) tree bark extract, anti-inflammatory, hyperglycemic rats, IL-1β gene expression, pancreas*

How to cite

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Highlights

1. Andalas *(Morus macroura Miq.)* tree bark extract significantly reduced IL-1β gene expression in hyperglycemic rats.
2. The 100 mg/kg of body weight dosage was most effective in suppressing pancreatic inflammation markers.
3. A 200 mg/kg of body weight dose showed the most significant reduction in fasting blood glucose levels
4. Higher doses (300 mg/kg of body weight) were less effective and showed potential for pro-inflammatory response.
5. The extract demonstrated potential as a natural anti-inflammatory and antihyperglycemic agent.

Introduction

Hyperglycemia, a disorder of metabolism characterized by elevated glucose in the blood, is a primary feature of numerous disorders, particularly diabetes mellitus (DM) (PERKENI, 2021). Chronic hyperglycemia results from impaired insulin secretion and/or action and is associated with long-term damage and dysfunction in multiple organs and tissues (Harreiter & Roden, 2023). Diabetes mellitus, particularly type 2, has become an important international health issue due to its severe effects and increasing occurrence. According to the International Federation of Diabetes (IDF, 2021), five hundred seventeen million people in the world through the onset of aging of twenty and seventy-nine are living with diabetes; by two thousand and thirty, that Figure is projected to rise to six hundred forty-three million, and by two thousand and fourty five, it will reach seven hundred eighty three million. With an estimated nineteen point five million cases, Indonesia has the fifth-highest prevalence of diabetes worldwide.

Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all diabetes cases worldwide. In Indonesia, the 2018 Basic Health Research (RISKESDAS) report noted a national prevalence of 8.5%, equating to approximately 20.4 million individuals. Moreover, diabetes is the third leading cause of death in Indonesia (6.7%), following stroke (21.1%) and coronary heart disease (12.9%) (Yarnita et al., 2023; Fajriah, 2022).

Emerging evidence suggests that T2DM is not only a metabolic disorder but also a chronic inflammatory disease. Hyperglycemia can activate the pathway that regulates NF-B, which in turn induces activated macrophages to create more cytokines that promote inflammation, including interleukin-1 beta and tumor necrosis factor alpha (Shita, 2015). By resulting in resistance to insulin, reduced production of insulin, and β-cell dysfunction, elevated Interleukin 1 beta levels worsen the progression of type 2 diabetes (Indrawati et al., 2023). As such, targeting IL-1β expression is a promising strategy in developing novel anti-inflammatory therapies for T2DM.

Although they are successful, conventional antidiabetic treatments, including insulin replacement, sulfonylureas, and metformin, are often linked to side effects like peripheral edema, weight gain, fluid retention, hypoglycemia, and gastrointestinal problems (Cortez-Navarrete et al., 2023). Interest in substitute, plant-based therapies that have therapeutic advantages with fewer adverse effects has increased as a result of these restrictions.

Morus macroura Miq., commonly known as the Andalas tree, is an endemic plant from West Sumatra with promising pharmacological potential. Triterpenoids, steroids, and dimerstilbenes are among the bioactive secondary metabolites found in its bark that have anticancer, anti-inflammatory, antibacterial, and antioxidant properties (Kurniawan et al., 2022). Although preliminary studies have indicated its antioxidant and anti-inflammatory potential, its specific effects on IL-1β expression under hyperglycemic conditions remain unexplored. The objective of this research is to evaluate the effects of *Morus macroura* Miq. bark extract on the expression of the Interleukin 1 beta gene transcript in the pancreatic tissue of rats with hyperglycemia.

Research Methods

Materials

Materials utilized in the investigation included alloxan monohydrate, Andalas (*Morus macroura* Miq) tree bark extract, ethanol 90% (Merck Pro Analysis), alcohol (Merck Pro Analysis), distilled water, TRIzol® reagent (Thermo Fisher Scientific, CA, USA), chloroform, isopropanol, RNAse Free Water, synthesis kit (Sensifast, Bioline, London,

UK), and male Wistar strain rats as materials and research subjects.

Instrumentation

The tools utilized included tweezers, scissors, Falcon tubes with capacities of 50 mL and 15 mL, measuring cup, gavage tube, oven, Erlenmeyer, filter paper, rotary evaporator, needle, centrifuge, and real-time PCR.

Procedure

This kind of research involves in vivo experiments and is a true experimental laboratory. The experimental animals were 24 *Rattus norvegicus* rats aged 2 months with weights ranging between 200 and 300 grams, all obtained from the same uniform source (Kristianingrum et al., 2017). Male Wistar strain rats were employed. Six groups were created from the rats, each of which had the following characteristics: treatment groups (P: hyperglycemic rats given Andalas bark extract *(Morus macroura Miq.)* according to earlier studies, at dosages of 100, 200, and 300 mg/kg body weight, adverse control (K-: typical rats), positive control (K+: hyperglycemic rats), and control (K: hyperglycemic rats treated with 500 mg metformin) (Kurniawan et al., 2022). The study's sample size was established using Federer's (1977) formula for experimental tests, which is as follows: (t-1) (n-1) ≥ 15, where (t) is the treatment group and (n) is the number of samples per treatment group. The sample size was determined using Federer's formula, as outlined in Equations (1a)–(1f).

$$(t-1)(n-1) \geq 15 \quad (1a)$$
$$(6-1)(n-1) \geq 15 \quad (1b)$$
$$5(n-1) \geq 15 \quad (1c)$$
$$5n-5 \geq 15 \quad (1d)$$
$$5n \geq 20 \quad (1e)$$
$$n \geq 4 \quad (1f)$$

From the results of the calculation of the large sample, each group consists of 4 rats. Sampling was carried out on a total sampling basis. This research includes the extraction of Andalas (*Morus mocroura* Miq.) tree bark, animal treatment test, RNA isolation, cDNA synthesis, gradient PCR amplification, Real-time PCR (RT-PCR), gene expression measurement, and statistical analysis.

1) Extraction of Andalas (*Morus mocroura* Miq.) tree bark

   The Andalas *(Morus macroura Miq.)* tree bark extract is taken from one place, namely in Mount Sago Payakumbuh, West Sumatra. The Andalas *(Morus macroura Miq.)* tree bark extract is baked at 50 °C. The result after drying in the oven is 200 grams of dried simplicia. The dried simplisia is then placed into the Erlenmeyer flask, and 90% ethanol solvent is added until the volume reaches 500 mL. Maceration is carried out for 3 days and continued with a filtering process with filter paper so that crude extract is obtained as a stock solution. After that, it is evaporated with a rotary evaporator for 1 day. The final result was 10 mL of Andalas tree bark extract.

2) Animal treatment test

   Male mice of the specific healthy 2–3 months old Wistar strain were placed in the Biomedical Laboratory of Andalas University under controlled conditions, such as room temperature of 25 °C and standard room humidity. Animals were permitted to eat and drink during the 12-hour/12-hour cycle of light and dark. The test animals were acclimated for seven days prior to the study. The FK UNAND ethics committee has approved the research protocol (No. 324/ UN.16.2/ KEP-FK/2024). A mouse model of hyperglycemia was created by administering aloxan at a concentration of 600 mg/10 ml of Aquades via intraperitoneal injection. To determine the rats' blood glucose levels, blood was extracted from their tail vein three days later. A glucose blood level of more than 250 mg/dL

during fasting in rats was considered hyperglycemia in this study (Utara, 2018). The administration of the extract in the treatment group was carried out orally every day for 2 weeks. After 2 weeks of treatment, the test animals were checked for fasting blood glucose levels after treatment, after which they were sacrificed and their pancreas was taken to measure the expression of the IL-1β gene.

3) RNA isolation

Total RNA from the pancreatic membrane tissue of all experimental groups was isolated using the reagent TRIzol(R) (Thermo Fisher Scientific, CA, USA) (Bharti et al., 2018). A homogenizer was used to homogenize the tissue, adding 1 milliliter of TRIzolTM reagent for every 50–100 milligrams of tissue in the piece of tissue. After adding 200 µL of chloroform, flip the tube over, and let it sit at room temperature for five minutes. After that, spin it for 15 minutes at 12,000 × g at 4 °C. The top or clear layer is transferred to a new sterile microtube. 2x isopropanol is added and re-incubated for 10 minutes at room temperature. After spinning at 12,000 × g for ten minutes at 4 °C, the pellets are washed immediately with 350 µL of 70% ethanol, and the resulting liquid is disposed of. The tube is turned back and forth and homogenized using a vortex slowly. The centrifuge returns at 7,500 × g at 4 °C for 5 minutes, then the supernatant is removed and the sample is vacuumed for 10 minutes. Once the vacuum is complete, resuspend the pellets in RNase-free water (25–40 µL, depending on the pellet volume). Then, the RNA was quantified and equalized at a concentration of 1000 ng (Sisca, 2021).

4) cDNA synthesis

The synthesis of cDNA is carried out using a synthesis kit (Sensifast, Bioline, London, UK). RNA is reverse-transcribed into cDNA (Wang et al., 2022).

5) Gradient PCR amplification

All PCR procedures were carried out within amplification conditions range for 40 amplification cycles, which included a predenaturation step for 3 minutes at 95 °C, a core cycle of 45 seconds at 94 °C, 30 seconds at 55 °C, 45seconds at 72 °C and 7 minutes of extension at 72 °C followed by a 5 minutes start of denaturation phase at 94 °C (Abid et al., 2019).

6) Real-time PCR (RT-PCR)

After cDNA synthesis is complete, the next step is RT-PCR using a gene-specific primer, which will be used according to the design and temperature optimization that have been carried out. Housekeeping genes include Glyceraldehyde-3-Phosphate Dehydrogenase (GADPH). Table 1 displays the main sequences for GADPH and IL-1β (Wang et al., 2022).

Table 1. Primer sequence of IL-1β and GADPH

Gene	Primer Type	Sequence (5'→3')
IL-1β	*Forward*	CCACCTTTTGACAGTGATGA
	Reverse	GAGATTTGAAGCTGGATGCT
GADPH	*Forward*	CATCATCCCTGCCTCTACTG
	Reverse	CCAAATTCGTTGTCATACCAGG

7) Gene expression measurement

The comparative Ct (ΔΔCt) method, as outlined by Livak and Schmittgen (2001), was the relative quantification method used in this investigation to quantify the expression of the IL-1β gene. Gene expression analysis was performed using the $2^{-\Delta\Delta CT}$ method (Equation (2a)–(2d)). This method involves the following steps. ΔCt (delta Ct) for each sample is measured as in Equation (2a). By comparing the target gene's Ct to that of a housekeeping gene that is consistently expressed, this normalization step accounts for differences in cDNA input. ΔΔCt (delta-delta Ct) is then calculated as in Equation (2c). At this stage, the experimental group's normalized Ct values are compared with those of the control sample. The relative gene expression value is finally determined using Equation (2d). This shows the target gene's fold change in expression between the experimental and control groups. This approach enables the comparison of gene expression between different treatment groups while accounting for technical variability through internal normalization.

$$\Delta C_{T\ experiment} = C_{T\ experiment\ target} - C_{T\ experiment\ housekeeping} \quad (2a)$$

$$\Delta C_{T\ control} = C_{T\ control\ target} - C_{T\ control\ housekeeping} \quad (2b)$$

$$\Delta\Delta C_{T\ experiment} = \Delta C_{T\ experiment} - \Delta C_{T\ control} \quad (2c)$$

$$\text{Comparison of gene expression levels} = 2^{-\Delta\Delta CT} \quad (2d)$$

8) Statistical analysis

All experimental groups were analyzed using a one-way ANOVA test using SPSS. If the value is $p<0.05$, then the value is considered significant (Bharti et al., 2018). The normality test for gene expression was carried out using the Shapiro-Wilk test and the homogeneity test with the Levene test. If the p-value was more than 0.05, the data were homogeneous and regularly distributed. Regularly distributed and homogeneous data were evaluated using ANOVA and Tukey's HSD Post Hoc-Test, whereas normal while homogeneous undistributed variables were analyzed using the Kruskal-Wallis test and Pairwise Comparisons test.

Results and Discussion

Effect of Andalas (Morus macroura Miq.) tree bark extract on the expression of IL-1β gene in the Pancreas of hyperglycemic rats

This study shows that the administration of Andalas *(Morus macroura Miq.)* tree bark extract may affect the expression of IL-1β gene transcripts in the pancreas of hyperglycemic mice. The results obtained in each research group are presented in Figure 1. After two weeks of treatment, the IL-1β gene's relative expression levels in the rats' pancreatic tissues returned to GADPH. P1, P2, P3 (Andalas extract 100, 200, 300 mg/kg OF BODY WEIGHT), K (metformin 500 mg/kg of body weight), K+ (hyperglycemic control), and K− (normal control).

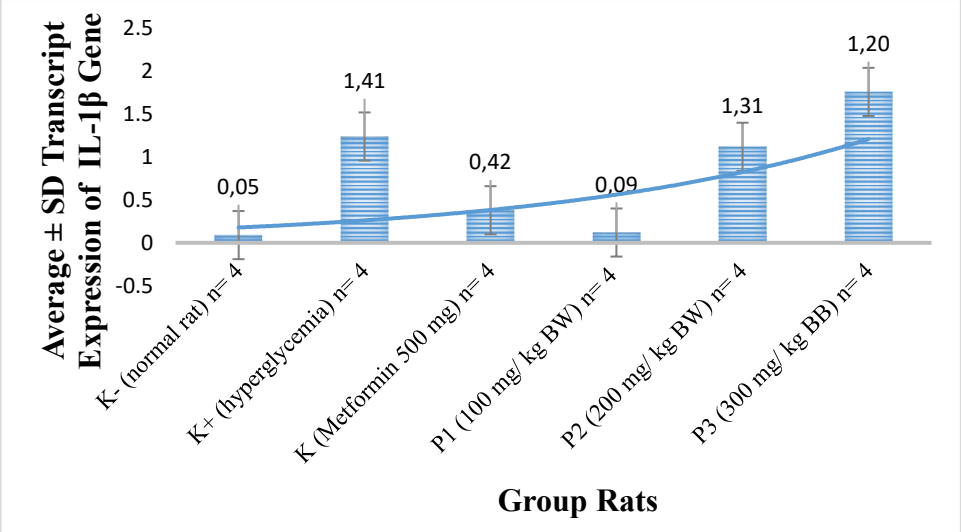

Figure 1. The IL-1β gene's relative expression levels in the rats' pancreatic tissues. (Note: The standard deviation is shown by error bars.)

The collected data were then subjected to analysis at a 95% confidence level and a significance level of 0.05 (p = 0.05). The data normalcy and comparability tests explain the analysis's findings. Following the measurement of the IL-1β gene concentration in each group, the data were statistically examined. In the control group, the results were 0.024, 0.281, 0.105, 0.074, 0.011, and 0.289, respectively. The Shapiro-Wilk Test was used to test for data normality. If the p-value was higher than 0.05, the data were considered normally distributed according to the Kruskal-Wallis nonparametric test. The Kruskal-Wallis test evaluates whether the average concentration of the IL-1β gene is statistically significant. When the p-value is less than 0.05, the difference value can be checked.

Table 2. Kruskal-Wallis test of the relative expression of IL-1β gene against GADPH in hyperglycemic rats after treatment with Andalas *(Morus macroura Miq.)* tree bark extract

Group	Average ± SD	% Decrease towards K+	*p*
K- (normal rat)	0.09 ± 0.05	-	
K+ (hyperglycemia)	1.24 ± 1.41	-	
K (Metformin 500 mg)	0.38 ± 0.42	69.4%	
P1 (100 mg/ kg of body weight)	0.12 ± 0.09	90.3%	0.032
P2 (200 mg/ kg of body weight)	1.12 ± 1.31	9.7%	
P3 (300 mg/ kg of body weight)	1.76 ± 1.20	-41.9%	

Table 2 shows the results of the Kruskal-Wallis test, which showed a significant variation within the average concentration of IL-1β gene in each study group after Andalas bark extract was administered ($p = 0.032$, $p<0.05$). Notably, certain groups, such as the positive control (K+) and the highest dose group (P3), exhibited high standard deviations (1.41 and 1.20, respectively), indicating substantial inter-individual variability in IL-1β expression. This could

be attributed to differences in metabolic responses to alloxan-induced hyperglycemia or individual differences in absorption and sensitivity to the extract. To validate these results and account for possible variability, more research using larger sample sizes and outlier analysis is advised. Pairwise comparisons were used for additional analysis to determine the significant difference in each group. Table 3 displays the outcomes of the pairwise comparisons. It showed the average relative expression of the IL-1β gene to GADPH in hyperglycemic rats after treatment with the bark of the Andalas *(Morus macroura Miq.)* tree.

Table 3. The relative expression of the IL-1β gene to GADPH in hyperglycemic rats after treatment with the *Morus macroura* Miq.) tree bark

Sample 1 - Sample 2	Sig.
K− - P1	0.802
K− - K	0.193
K− - K+	0.147
K− - P2	0.024*
K− - P3	0.004*
P1 - K	0.293
P1 - K+	0.230
P1 - P2	0.045*
P1 - P3	0.009*
K - K+	0.881
K - P2	0.342
K - P3	0.121
K+ - P2	0.423
K+ - P3	0.161
P2 - P3	0.548

Caption : *Significant difference ($p<0.05$)

According to Table 3, the normal rat group (K-) with a dose of 200 mg/kg of body weight(P2), normal rats (K-) with a dose of 300 mg/kg of body weight(P3), a dose of 100 mg/kg of body weight(P1) with a dose of 200 mg/kg of body weight(P2), and a dose of 100 mg/kg of body weight(P1) with a dose of 300 mg/kg of body weight(P3) were the groups that differed significantly from the other groups. Despite high variability within groups (e.g., SD = 1.41 in K+), the comparison revealed statistically significant differences between P1 and P3 ($p = 0.009$), and between K− and P3 ($p = 0.004$), suggesting a dose-dependent, but non-linear, anti-inflammatory response to Andalas bark extract.

The results showed that the average IL-1β gene expression dropped in the metformin 500 mg groups (K), P1, and P2 compared to the control group that was positive after ingesting Andalas *(Morus macroura Miq.)* tree bark extract. The extract dosage group receiving 100 mg/kg body weight (P1) had the lowest IL-1β level, at 90.32 percent, compared to metformin 500 mg, which reduced IL-1β gene expression by 69.35%. This implies that a dosage of 100 mg/kg body weight of the bark extract from Andalas trees is more efficient at reducing the expression of the IL-1β gene. The average IL-1β gene expression did not drop in the third treatment group. The reduction in IL-1β gene expression suggests that Andalas tree bark extract plays a role in inhibiting inflammation in hyperglycemia induced by alloxan and has an activity similar to the comparative drug metformin. Metformin is able to mimic calorie restriction through AMP kinase activation and can prolong lifespan and health in several rat species.

Precision in using the extract dosage is key to maximizing benefits while minimizing side effects. Each herbal extract has a safe and effective dosage range. Exceeding the optimal dose can endanger health and potentially become toxic to the body. Like synthetic medications, herbal extracts can cause adverse effects if consumed excessively. Doses that are too low will not produce the desired effect, while doses that are too high can become dangerous toxins (Bucciantini et al., 2021).

When compared to two other doses, the 100 mg/kg body weight dose of Andalas *(Morus macroura Miq.)* tree bark extract produced the best results in this study. At

this dosage, IL-1β gene expression showed the most significant reduction, indicating strong anti-inflammatory potential. However, when the dosage was raised to 200 mg/kg of body weight, there was a decline in effectiveness for suppressing IL-1β gene expression. Although a reduction still occurred, the results were not as good as those at the 100 mg/kg body weight dose. These findings suggest that excessively high doses can diminish the expected therapeutic effect. More concerningly, when the dosage of the extract was raised to 300 mg/kg of body weight, no reduction in IL-1β gene expression was observed at all.

Several studies have reported that high doses of certain anti-inflammatory agents, including plant-based extracts, may lead to diminished efficacy and potential toxic effects. For example, overdosing on herbal extracts decreased their anti-inflammatory effects and introduced symptoms of organ stress and cellular toxicity, according to Shafiq et al. (2021) and Mahmoud et al. (2021). While the exact mechanisms are still under investigation, it is suggested that exceeding the optimal therapeutic window may disturb the delicate balance of cytokine regulation and cellular homeostasis.

These findings underscore the importance of using precise dosages when utilizing herbal compounds. Doses that are too low may lack effectiveness, while excessively high doses can be harmful and eliminate therapeutic benefits. Researchers concluded that 100 mg/kg body weight of Andalas tree bark extract represents the optimal dose for achieving maximal anti-inflammatory effects through IL-1β gene expression suppression, without inducing adverse side effects. This discovery provides a foundation for further research in developing herbal products based on Andalas tree bark extract, illustrating a common pattern of optimal dose effects, reduced efficacy at higher doses, and toxicity at extremely high doses. This pattern is frequently observed in other herbal extract studies, despite variations in plant species.

In a study by Y. Shafiq et al. (2021) investigating Nerium oleander extract's effects on IL-1β production and mRNA expression in a carrageenan-induced paw edema rat model, researchers found that at specific doses, the extract significantly reduced IL-1β levels and mRNA expression. However, at higher doses, the effects became less effective and showed signs of toxicity. IL-1β mRNA expression and IL-1β production were significantly reduced at dosages of 250 mg/kg and 500 mg/kg. At higher doses, the anti-inflammatory effects diminished, and cellular toxicity signs emerged (Shafiq et al., 2021).

The study on mango ginger root extract by Mahmoud et al. (2021): In the inflammatory bowel model rats, the extract decreased oxidative stress and inflammation at 100–200 mg/kg body weight. However, at >500 mg/kg body weight, protective effects disappeared, and liver and kidney toxicity signs emerged. Bucciantini et al. (2021) tested olive leaf extract as an anti-inflammatory agent. They found optimal effects at 50-100 mg/kg for inhibiting inflammation, but doses >200 mg/kg increased inflammation and damaged kidney function (Bucciantini et al., 2021).

Recent studies have also highlighted the role of formulation and phytochemical content in enhancing the antioxidant and therapeutic effects of plant-based compounds. For example, curcumin encapsulation strategies (Wiratantri et al., 2024) and phytochemical analysis of *Myristica fragrans* (Saputri et al., 2024) demonstrated strong antioxidant and potential anticancer activity, supporting the relevance of natural products like *Morus macroura* Miq. in inflammation control.

The reduction in IL-1β gene transcript expression is caused by Andalas *(Morus macroura Miq.)* tree bark extract, containing various secondary metabolites such as triterpenoid derivatives, steroids, dimer stilbenes, and others, with anti-inflammatory compound activities that can suppress NF-κB signaling due to hyperglycemic conditions, thus inhibiting NLRP3 activation and IL-1β gene expression. Cells exhibit the NLRP3 inflammasome, a protein complex that recognizes danger signals and triggers the creation and maturation of active caspase-1, which releases cytokines such as IL-18, IL-33, and IL-1β. It is activated by various stimuli, particularly the detection of metabolic signals, such as increased extracellular glucose, a crucial manifestation of diabetes mellitus. Procaspase-1, ASC, and NLRP3 make up the inflammasome. Its creation leads to caspase-1 activation through self-cleavage, resulting in the development of IL-1β and IL-18, and initiating piroptosis, a GSDMD-mediated form of inflammatory cell death (Sun et al., 2020).

To activate the NLRP3 inflammasome, two signals are required. The activation of TLR4 stimulates the production of pro-IL-1β and NLRP3 by initiating the NF-κB signaling pathway. The production of ROS, cathepsin from lysed lysosomes, and K+ efflux provide a secondary trigger for activating the NLRP3 inflammasome. As a result, the activation of caspase-1 is produced, which leads to the maturation of IL-1β. Small molecules such as COPs and POPs, cells, cytokines, autophagy, NO, and CO can have a negative impact on NLRP3 inflammasome activation. Hyperglycemia develops as a result of beta cell malfunction and death brought on by IL-1β (Sun et al., 2020). NF-kB inhibitor (IkBa) is a protein that inhibits NF-kB, substantially stopping inflammation development by preventing its degradation. Through a multi-step process involving the activation of pro-inflammatory signals, the primary sources of IL-1β are dendritic cells, macrophages, and monocytes. (Fauza and Febriawan, 2023).

The NF-kB suppression activity of Andalas tree bark extract explains its beneficial role in reducing biological membrane and tissue damage caused by IL-1β-induced inflammation. The extract contains various anti-inflammatory compounds, consistent with previous research findings, 2-arylbenzofuran derivatives with anti-inflammatory, antiviral, and antitumor properties. Specific compounds like morasin M have been identified with the capability to stop cancer cell growth and provide anti-inflammatory effects (Monita, 2022). Based on the research, the study's hypothesis is accepted, confirming the extract's influence on reducing IL-1β gene transcript expression in hyperglycemic rat pancreas.

Effect of Andalas (Morus macroura Miq.) tree bark extract and metformin treatment on rats' blood glucose levels

This study demonstrates that administering hyperglycemic rats with extract from the bark of the Andalas tree *(Morus macroura Miq.)* can alter their blood glucose levels. In Figure 2, the findings from each research group are displayed. The lowest glucose was found in P2 (200 mg/kg of body weight), and there were no variations in fasting glucose levels.

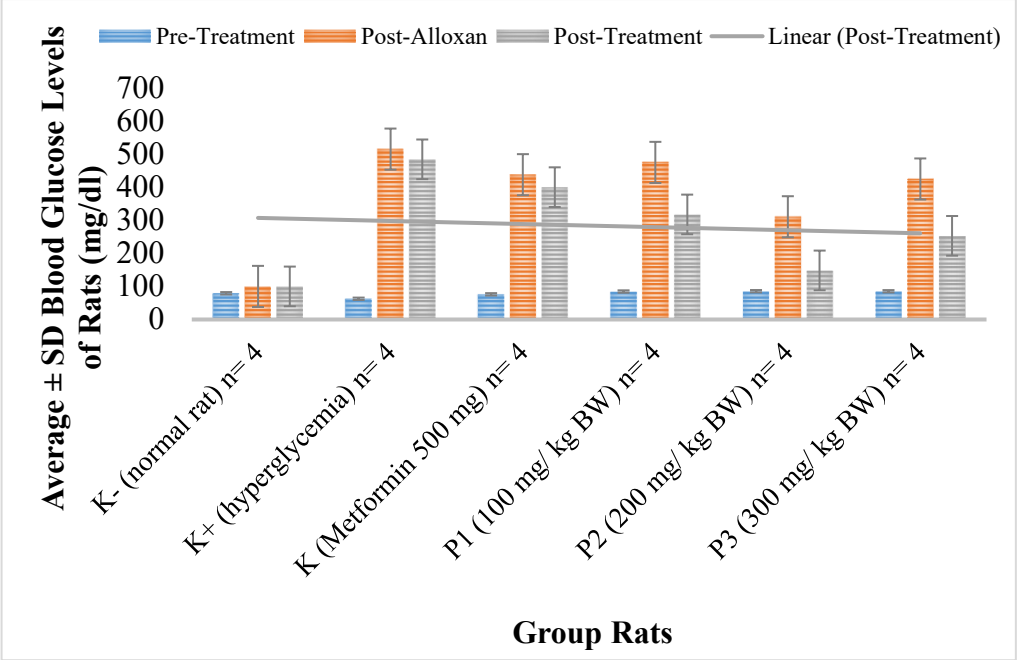

Figure 2. Rats' average fasting blood glucose levels after two weeks of therapy. (Note: SD is indicated by error bars.)

Table 4. Kruskal-Wallis test on average fasting blood glucose levels of rats for 2 weeks of treatment with Andalas *(Morus macroura Miq.)* tree bark extract

Group	Average ± SD	*p*
K- (normal rat)	100.25 ± 25.44	0.012
K+ (hyperglycemia)	485.25 ± 157.89	
K (Metformin 500 mg)	401.00 ± 136.35	
P1 (100 mg/ kg of body weight)	318.25 ± 218.91	
P2 (200 mg/ kg of body weight)	148.75 ± 33.12	
P3 (300 mg/ kg of body weight)	253.50 ± 23.41	

According to Table 4, the results of the Kruskal-Wallis test showed a significant variation in the mean fasting blood glucose levels of rats in each research group after administration of Andalas bark extract, with a p-value of 0.012 ($p < 0.05$). The high SD values in P1 (218.91) and K+ (157.89) groups suggest heterogeneous responses to treatment or induction methods. Variability may reflect inconsistent induction of hyperglycemia or differential sensitivity to treatment, warranting further investigation. To find out the difference in significance in each group, further analysis was carried out using Pairwise Comparisons. The results of Pairwise Comparisons are presented in Table 5.

Groups K with P3, K with K-, K with K+, and P2 with K+ were the ones that differed significantly from the other groups, as indicated in Table 5. According to the research findings, the therapy that was given to the rats caused their blood glucose levels to drop. Rat groups containing hyperglycemic + metformin 500 mg (K), hyperglycemic + Andalas tree *(Morus macroura Miq.)* bark extract at 100 mg/kg body weight (P1), hyperglycemic group + extract dose 200 mg/kg body weight (P2), and hyperglycemic rat group + extract dose 300 mg/kg body weight (P3) all showed a reduction in levels of blood glucose.

After two weeks of treatment, blood glucose levels changed, according to the mean Table of blood glucose level

accumulation. At week two following the treatment of Andalas *(Morus macroura Miq.)* tree bark extract, variance analysis results demonstrated a substantial (p = 0.012) decrease in the levels of blood glucose of hyperglycemic rats. With 200 mg/kg body weight of Andalas *(Morus macroura Miq.)* tree bark extract, the hyperglycemic group experienced the greatest blood glucose reduction (P2), 52.20% (p=0.016). This suggests that the 200 mg/kg body weight extract was more successful in lowering rat blood glucose levels than metformin 500 mg, which decreased blood glucose levels by 8.60%.

Table 5. Results of pairwise comparisons analysis on average fasting blood glucose levels of rats for 2 weeks of andalas tree bark extract treatment *(Morus macroura Miq.)*

Sample 1 - Sample 2	Sig.
K-P2	0.342
K-P1	0.099
K-P3	0.033*
K-K-	0.006*
K-K+	0.001*
P2-P1	0.483
P2-P3	0.239
P2-K-	0.076
P2-K+	0.016*
P1-P3	0.634
P1-K-	0.282
P1-K+	0.089
P3-K-	0.548
P3-K+	0.220
K--K+	0.532

Note: * Difference that is significant (p<0.05)

The unique way that metformin lowers blood glucose levels may be the reason for the blood glucose decrease in the hyperglycemic group that received treatment with it (K). Metformin's mechanisms include direct glycolysis stimulation in peripheral tissues with increased glucose elimination from blood, reducing liver gluconeogenesis, slowing glucose absorption in the intestine, reducing plasma glucagon levels, and increasing insulin binding to insulin receptors. Metformin's mechanism for lowering blood glucose is not dependent on functional pancreatic β cells (Prameswari and Widjanarko, 2014).

Blood glucose reduction with Andalas *(Morus macroura Miq.)* tree bark extract therapy can be caused by bioactive compounds contained in the extract that can prevent oxidation in pancreatic β cells, thereby minimizing damage. Bioactive compounds in the extract include triterpenoid derivatives, steroids, dimerstilbene, and others.

Triterpenoid derivatives can lower blood glucose levels in alloxan-induced rats by increasing insulin secretion from the pancreas. The action mechanism of terpenoid compounds as anti-diabetics is stimulating insulin release and helping glucose absorption by stimulating GLUT-4 in cells. Triterpenoids function as insulin level suppliers in the body and help the pancreas increase insulin production, thereby increasing the amount of insulin needed by the body to bind blood sugar levels, thus lowering blood sugar and insulin requirements.

To prevent or treat diabetes mellitus, health experts generally use medications that cause hypoglycemic effects and insulin-increasing effects. These types of drugs are usually the first prescribed to treat diabetes mellitus.

The Andalas *(Morus macroura Miq.)* tree bark extract, containing triterpenoids with hypoglycemic activity, can reduce blood sugar levels, consequently reducing glucose levels in patients with diabetes mellitus. This is consistent with studies by Amin Zakaria et al. (2019), which discovered that triterpenoids in fig leaf tea may help people with diabetes mellitus lower their blood sugar levels.

The differences in effective doses between IL-1β reduction and glucose reduction by Andalas tree bark extract can be explained by several factors. Different action mechanisms involve anti-inflammatory pathways for IL-1β

reduction, while glucose reduction is related to anti-diabetic effects or increased insulin sensitivity. Target sensitivity, receptors, or signaling pathways involved in IL-1β regulation are more sensitive to active compounds in the extract, whereas glucose reduction mechanisms require higher concentrations to achieve significant effects. Pharmacokinetics, differences in absorption, distribution, or metabolism of active compounds at different doses can affect effective concentration in target tissues. The extract's complexity means plant extracts contain various compounds, some more effective in reducing IL-1β, while others are more effective in reducing glucose. Individual variations and experimental conditions, differences in study design, treatment duration, or experimental animal characteristics can also influence results (Prameswari and Widjanarko, 2014).

IL-1β (inflammation marker) reduction is more easily detected with small changes compared to blood glucose level changes. Anti-inflammatory effects have a lower threshold for significant visibility compared to glucose reduction effects. Anti-inflammatory effects are more direct and require interaction with fewer body systems compared to glucose regulation, which involves various organs and hormones (Prameswari and Widjanarko, 2014).

Effect of Andalas (Morus macroura Miq.) tree bark extract treatment on the body weight of hyperglycemic rats

This study shows that the administration of Andalas *(Morus macroura Miq.)* tree bark extract can affect the weight of hyperglycemic rats. The results obtained in each research group are presented in Figure 3. It shows that the weight among the rats in each research group increased after receiving Andalas *(Morus macroura Miq.)* tree bark extract. According to the study, giving hyperglycemic rats bark extract from the Andalas tree *(Morus macroura Miq.)* can affect their body weight. Following two weeks of treatment, body weight assessments for each treatment group (K-, K+, K, P1, P2, and P3) revealed an increase over the previously prescribed regular diet. The P2 therapy group experienced the largest percentage rise (32.57%), which was substantially different from the other groups.

The differences in body weight percentage changes in hyperglycemic rats were caused by varying metabolic responses to hyperglycemia-inducing factors. Some rats experienced mild hyperglycemia, while others had severe hyperglycemia. Mild hyperglycemia is related to the stability of genetic and internal rat conditions, with pancreatic β cells being slowly damaged by receptors. The production of ROS by alloxan causes selective β-cell necrosis, partial destruction of pancreatic islet β cells, and disruption of the quantity and quality of insulin generated by β cells, all of which contribute to severe hyperglycemia (Eky Nursia et al., 2020).

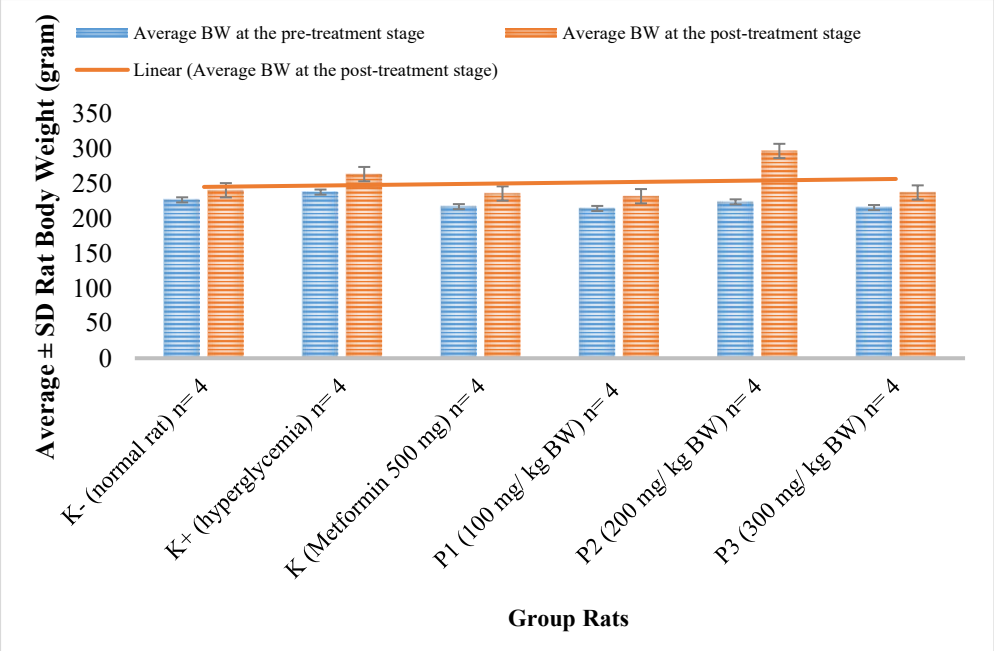

Figure 3. Effect of Andalas *(Morus macroura Miq.)* tree bark extract treatment on the body weight alteration of hyperglycemic rats

This effect is attributed to the Andalas (Morus macroura Miq.) tree bark extract, which contains active antioxidant compounds that play a crucial role in enhancing the body's metabolic system, thereby causing the rats' body weight to increase. Looking at the percentage presented in Table 5, the body weight increase in P1 is closer to K- compared to P2 and P3. This proves that the lowest dose (100 mg/kg body weight) in P1 can repair metabolic cell damage. P2 and P3 treatments showed higher body weight percentage increases compared to KN, possibly because the administered doses were higher than those in P1, thus accelerating pancreatic β cell recovery and addressing insulin secretion metabolic process abnormalities more quickly.

Overall, the body weight percentage increase in P1, P2, and P3 exceeded K-, which was caused not only by pancreatic β-cell repair but also by the hyperglycemic condition of the rats, leading to unstable body metabolism. Each rat's body response differs, causing variations in percentage Figures across groups. These study results are consistent with Eluihike's (2018) research, which found that rats fed extracts containing antioxidants (tannins) had much higher body weights than the control group. Rats' loss of adipose tissue fat and muscular tissue's catabolism of amino acids were the causes of this. Rats felt hungry more quickly as a result of structural protein breakdown, which increased their food intake (Eky Nursia et al., 2020).

The Andalas *(Morus macroura Miq.)* tree bark extract contains various phytochemical compounds that influence body weight increase in hyperglycemic rats. Several studies mention that the extract contains triterpenoid derivatives, steroids, dimerstilbene, and others with compounds exhibiting anti-inflammatory, antioxidant, antimicrobial, antitumor, and antidiabetic activities. The active antioxidants are essential for enhancing the body's metabolic processes.

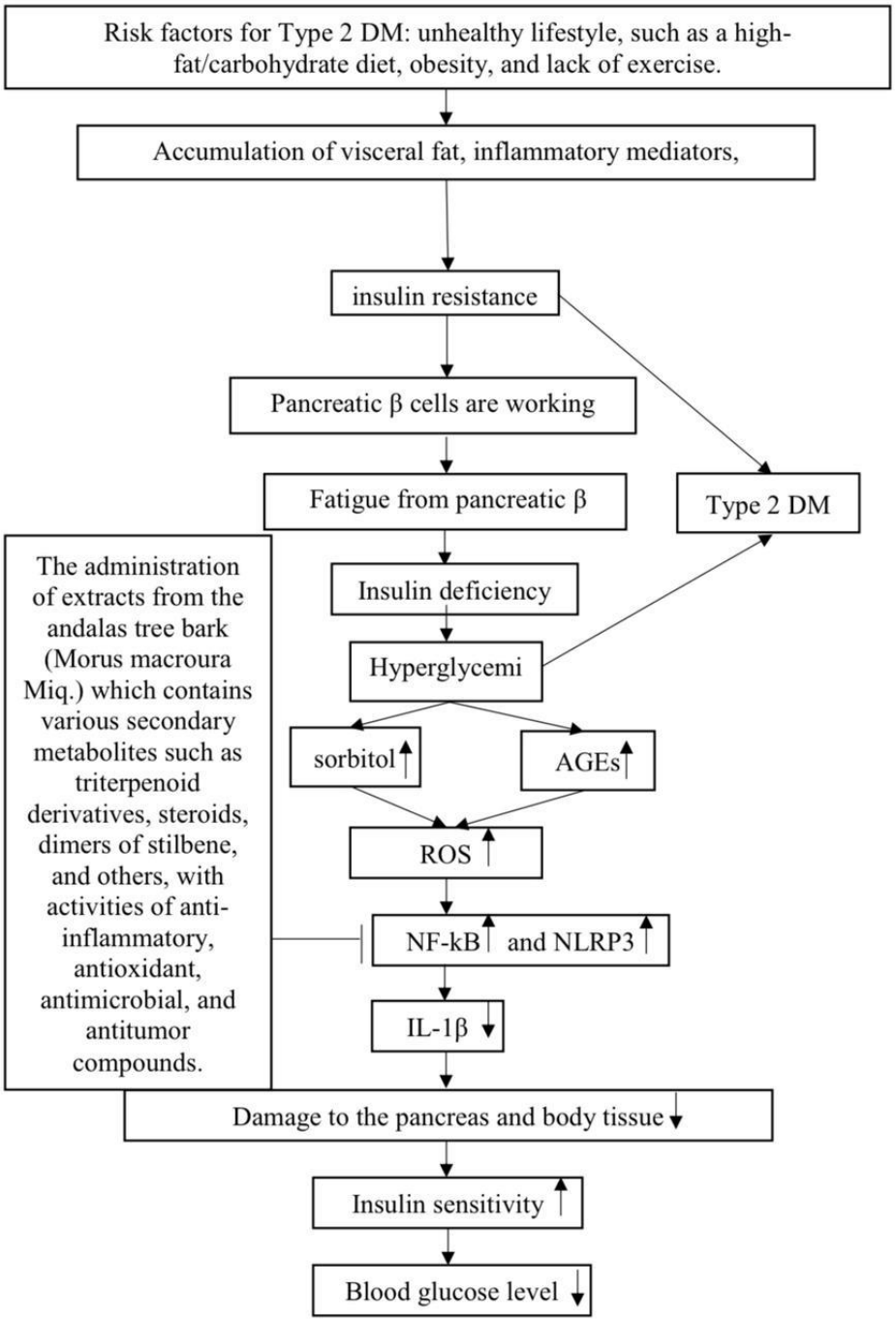

Figure 4. A diagram outlining the mechanism by which Andalas tree bark extract can lower blood sugar in hyperglycemic rats and suppress IL-1β gene expression

Limitations of the study

This study has several limitations that should be acknowledged. First, the generalizability of the findings is limited by the small sample size (n = 4 per group), which may also lower the statistical power to detect minute changes between groups. Second, only male Wistar rats were used, which may exclude potential sex-specific responses to the extract, particularly considering known differences in metabolic and hormonal profiles between male and female animals. Third, the intervention period was relatively short (14 days), which may not be sufficient to capture the long-term effects or potential toxicities of *Morus macroura* Miq. bark extract. Future studies should consider longer treatment durations, include both sexes, and increase the number of animals

per group to strengthen the reliability and applicability of the results.

Conclusions

Based on the research that has been conducted, it can be concluded that the administration of Andalas *(Morus macroura Miq.)* tree bark extract has a significant effect on transcriptional expression of IL-1β gene in the pancreas of hyperglycemic rats. A dosage of 100 mg/kg of body weightwas shown to be effective in reducing the expression of IL-1β gene transcripts. However, there was a decrease in effectiveness at a dosage of 200 mg/kg of body weight, and no effect was observed at a dosage of 300 mg/kg of body weight. Meanwhile, a dosage of 200 mg/kg of body weightshowed the best results in lowering glucose levels and increasing the weight of the rats.

Author Contributions

YA conceptualized and supervised the study. **WJH** performed the experiments and collected the data. **WJH** and **YA** conducted data analysis and visualization. **WJH** prepared the original draft. **WJH** and **YA** reviewed and edited the manuscript. All authors contributed and agreed to the final version of the manuscript.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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