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The Effect of Jeruju Leaf Brew (*Acanthus ilicifolius* L) on HOMA-IR and TNF-α in White Rats Induce by STZ-NA

***Munifa Majdiyyah[1], Ade Chairina[1], Diana Nur Afifah[1,2], Ahmad Syauqy[1]**

[1]Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia 50275. [2]Center of Nutrition Research (CENURE), Diponegoro University, Semarang, Central Java, Indonesia 50275
*Email: munifamajdiyyah@gmail.com. DOI: 10.31964/mltj.v11i1.648

Abstract: The International Diabetes Federation (IDF) has reported a consistent global rise in the prevalence of type 2 diabetes mellitus (T2DM). Conventional T2DM treatments such as metformin, sulfonylureas, and insulin therapy are effective. Still, they can lead to side effects, including gastrointestinal disturbances, hypoglycemia, renal impairment, weight gain, and complications associated with injection procedures. The herbal remedies are considered a natural alternative with fewer adverse effects. *Acanthus ilicifolius* (Jeruju) contains antioxidant compounds such as flavonoids, alkaloids, and saponins and may serve as a complementary therapy. This study investigated the effect of jeruju leaf brew at 6 ml/kg/day and 12 ml/kg/day on tumor necrosis factor alpha (TNF-α) and homeostatic model assessment of insulin resistance (HOMA-IR) levels. Using a true experiment with a randomized post-test only with a control group design, 30 rats were divided into five groups (K0, K−, K+, P1, and P2). After 28 days of intervention, groups P1 and P2 showed significant reductions in TNF-α (7.80 pg/ml and 6.94 pg/ml, respectively) and HOMA-IR (3.75 and 3.28, respectively). These findings indicate that a higher dose of jeruju leaf brew results in greater reductions in TNF-α and HOMA-IR levels in T2DM rats.
Keywords: *Acanthus ilicifolius*; homeostatic model assessment of insulin resistance (HOMA-IR); jeruju; tumor necrosis factor alpha (TNF-α); type 2 diabetes mellitus (T2DM).

INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder characterized by hyperglycemia with blood glucose levels ≥ 126 mg/dL (American Diabetes Association, 2015; Petersmann et al., 2019). This condition arises due to impaired insulin secretion, insulin resistance, or both, along with disturbances in nutrient metabolism. Type 2 diabetes mellitus (T2DM) is the most prevalent form, accounting for approximately 90% of all global diabetes cases. According to the International Diabetes Federation (IDF), the global prevalence of T2DM continues to rise annually; in 2021, over 537 million cases of T2DM were reported, and this number is projected to reach 783 million by 2045 (Mahgoub et al., 2023; Patel et al., 2022). Indonesia is one of the countries significantly affected, with the 2023 Indonesian Health Survey (SKI) showing an increase in T2DM cases, reaching a prevalence of 11.7%, predominantly among the productive age population (Oktora & Butar Butar, 2022).

T2DM is associated with impaired insulin secretion and insulin resistance due to pancreatic β-cell dysfunction, including reduced pulsatility and biphasic insulin secretion, decreased glucose sensitivity, and reduced β-cell mass (Adhikari, 2021). In addition to insulin resistance, which can be assessed using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index, hyperglycemia also triggers

Corresponding Author: Munifa Majdiyyah
Department of Nutrition Science, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia 50275.
Email: munifamajdiyyah@gmail.com

oxidative stress, a condition marked by an imbalance between free radicals and the body's ability to neutralize them with antioxidants. Oxidative stress damages pancreatic cells decreases insulin secretion and elevates systemic inflammation marked by increased TNF-α levels. High TNF-α levels exacerbate insulin resistance by promoting ROS production, creating a pathological feedback loop (Martin et al., 2016). Long-term complications effect of T2DM include cardiovascular disease, the leading cause of death among diabetic patients, severely impacting quality of life and increasing mortality and morbidity (Adhikari, 2021).

Commonly, T2DM conventional management involves lifestyle modifications, pharmacotherapy, and insulin therapy (Deutschländer et al., 2011). Despite their effectiveness, long-term use of medications such as metformin and sulfonylureas may result in side effects, including diarrhea, nausea, bloating, hypoglycemia, and renal dysfunction (T. T. L. Lee et al., 2022; Riwu et al., 2015). Continuous insulin therapy may also cause hypoglycemia, weight gain, and injection-related reactions (Rahman & Islam, 2024; Udayani et al., 2021). There has been a growing interest in herbal therapies as complementary treatments in a few decades due to their perceived natural benefits and lower side effect profile (Abdullah, 2024).

One of the herbal plants with potential as a complementary therapy is *Acanthus ilicifolius*, locally known as Jeruju (Ni Made Sugi Pradnyasuari & Anak Agung Gede Rai Yadnya Putra, 2023). This plant contains bioactive compounds with anti-inflammatory, antidiabetic, and antioxidant properties, helpful in managing insulin resistance and oxidative stress, two key aspects of T2DM pathophysiology (Made Sugi Pradnyasuari & Agung Gede Rai Yadnya Putra, 2023; Natarajan et al., 2024). Jeruju is known as a mangrove type and is abundant in coastal areas of Indonesia, including Ayah Beach, Kebumen, and Central Java. The availability of jeruju in the region is relatively plentiful, but remains underutilized by local communities, who traditionally use it for tea or snacks (Verma et al., 2020). Nevertheless, the local community has long held a traditional belief that the leaves possess medicinal properties for treating T2DM. This belief is supported by scientific research indicating that jeruju leaves contain phytochemical compounds and exhibit significant pharmacological activity. Scientific studies have shown jeruju leaves to contain flavonoids, alkaloids, and saponins with antidiabetic, anti-inflammatory, hepatoprotective, antiosteoporotic, anticancer, and antibiotic properties (Arnetta Deandra et al., 2024; Nurfitri et al., 2019; Sadeer & Mahomoodally, 2022).

The flavonoid compounds quercetin and fisetin have been proven to inhibit key components of the MAPK pathway, thereby suppressing NF-κB activation by reducing the phosphorylation of IκB and p65, thereby lowering TNF-α levels (Zhong et al., 2022; Zu et al., 2021). Fisetin and saponins enhance GLUT-4 translocation to the plasma membrane, promoting glucose uptake into muscle and adipose tissues, contributing to the improvement of blood glucose levels and insulin resistance (HOMA-IR) (Ashari & Nurinda, 2021; Azhar et al., 2022; Gayathri & Kavya P, 2023). Alkaloids help reduce oxidative stress and improve insulin secretion and sensitivity (Adhikari, 2021; Kumari & Jain, 2012). In the preceding decade, 20 scientific articles have investigated the potential of *Acanthus ilicifolius* (jeruju) leaves. The extant studies employed various extract preparations, with 14 focusing primarily on the analysis of phytochemical constituents and general pharmacological potential in the health sector (Arnetta Deandra et al., 2024; Batubara et al., 2022; Biswas et al., 2019; Islam et al., 2024; Made Sugi Pradnyasuari & Agung Gede Rai Yadnya Putra, 2023; Natarajan et al., 2024; Sadeer & Mahomoodally, 2022; Sravani et al., 2024; Ulya et al., 2022; Vani & Manikandan, 2018; Verma et al., 2020; Vira Arunita et al., 2023; Wulansari et al., 2024;

Zhang et al., 2022). The remaining six studies were conducted on animal models. However, they did not specifically examine inflammatory parameters or insulin resistance (Gayathri & Kavya P, 2023; Karim et al., 2021; Nurfitri et al., 2019; Rizeki et al., 2020a; Widiastuti et al., 2021; Zohora et al., 2023). This finding suggests a research gap, particularly concerning the efficacy of jeruju leaf brew in managing type 2 diabetes mellitus. Therefore, this study employs an infusion preparation, which is considered more practical, applicable, and aligned with traditional consumption practices.

The present study employs a combinative intervention strategy, encompassing metformin administration at a dosage of 45 mg/kgBB/day and jeruju leaf brew at 6 ml/kgBB/day and 12 ml/kgBB/day. This approach is utilized in male white rats induced with STZ-NA. TNF-α parameters were selected as indicators of inflammation, and HOMA-IR was chosen as an indicator of insulin resistance, considering that both are the main parameters that play an essential role in the pathophysiology and progressivity of T2DM. It is hypothesized that this combination therapy will significantly reduce TNF-α levels and HOMA-IR more significantly than single therapy. The mechanism of action of metformin is by inhibiting the gluconeogenesis process and increasing cell sensitivity to insulin. Meanwhile, the bioactive compounds in jeruju leaf brew contribute through antioxidant and anti-inflammatory activities that support the improvement of metabolic conditions. Consequently, jeruju leaf brew emerges as a promising adjuvant therapy, potentially enhancing the efficacy of conventional metformin therapy in the comprehensive management of T2DM.

MATERIALS AND METHODS

This study employed a true experimental method using a randomized post-test with a control group design. The experiment was conducted at the Center for Food and Nutrition Studies Laboratory, Universitas Gadjah Mada (PSPG PAU UGM). Ethical approval was granted by the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University (Approval No. 093/EC-H/KEPK/FK-UNDIP/IX/2024). The study sample consisted of thirty male Wistar rats *(Rattus norvegicus)*, aged eight weeks and weighing 150–200 grams. The independent variable was jeruju leaf brew, while the dependent variables were HOMA-IR and TNF-α levels.

Research Materials

The jeruju leaves (*Acanthus ilicifolius*) utilized in this study were obtained from the Essential Ecosystem Area (EAA) of Muara Kali Ijo, Kebumen, Central Java. Before this study, the plant species *Acanthus ilicifolius* had been identified through a rigorous profiling process. The jeruju leaf brew was prepared using a modified version of the Japanese Green Tea Process (JGTP) in six stages: harvesting, spine removal and washing, blanching, cooling, drying, and pulverization. Mature leaves were sorted, cleaned, and blanched for 75 seconds. After soaking in water and separating the leaf blades from the midribs, the leaves were dried using a food dehydrator at 80°C for 2.5 hours and then ground with a chopper to achieve the desired consistency (Anjani et al., 2020).

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KO: Normal control group
K-: Induced with STZ 45 mg/kg and NA 110 mg/kg (intraperitoneal)
K+: Induced with STZ 45 mg/kg and NA 110 mg/kg + metformin 45mg/kg/ day (per oral), 28 days

P1: Induced with STZ 45 mg/kg and NA 110 mg/kg + metformin 45mg/kg/ day and jeruju leaf brew 6 ml/kg/day (per oral), 28 days
P2: Induced with STZ 45 mg/kg and NA 110 mg/kg + metformin 45mg/kg/ day and jeruju leaf brew 12 ml/kg/day (per oral), 28 days

Jeruju Leaf Brew Dosage

Two grams of jeruju leaves were infused in 100 ml of hot water (90°C) and steeped for 10 minutes before being administered. Dosage calculations were based on (Mahammad, 2023), who administered 6 ml/kg of *Cnidoscolus aconitifolius* leaf tea as an adjuvant to metformin in T2DM management. Therefore, this study used two concentrations: 6 ml/kg/day and 12 ml/kg/day.

Metformin Dosage

Metformin is the first-line oral medication recommended for T2DM (Pan et al., 2016; Za'abi et al., 2021). This study was administered based on a human-to-rat dose conversion. With a human dose of 500 mg and a conversion factor of 0.018, the rat dose was calculated as 45 mg/kg.

HOMA-IR and TNF-α Check

This analysis uses blood samples collected via retro-orbital plexus using 2cc hematocrit capillary tubes after an 8-10 hour fasting period. HOMA-IR was calculated using fasting blood glucose and insulin levels with the formula: HOMA-IR = (Fasting Glucose × Fasting Insulin)/405. This value can describe the sensitivity of insulin receptors in peripheral tissues. Fasting blood glucose was measured using the Glucose Oxidase-Peroxidase Aminoantipyrine (GOD-PAP) method. Insulin levels were measured using a Fine Test Rat INS (Insulin) ELISA kit, and TNF-α levels were analyzed using a Rat TNF-α ELISA kit.

Statistical Analysis

This data normality was tested using the Shapiro-Wilk test due to the small sample size (<50). One-way ANOVA test followed by Bonferroni post hoc tests were used to determine group differences. Statistical significance was defined at $p \leq 0.05$. All data analyses were performed using SPSS version 25.

RESULTS AND DISCUSSION

According to previous research, jeruju leaves contain several secondary metabolites, including flavonoids, alkaloids, and saponins (Arnetta Deandra et al., 2024; Vani & Manikandan, 2018). These compounds are known for their antioxidant, anti-inflammatory, antidiabetic, anticancer, and antimicrobial activities (Natarajan et al., 2024; Pothiraj et al., 2021). Antioxidants inhibit or neutralize the harmful effects of free radicals and oxidative stress. They donate electrons to stabilize free radicals, preventing further cellular damage. The antioxidants are essential in depressing oxidative stress and preventing many diseases (Balta et al., 2022; Blokhina, 2003; Sharifi-Rad et al., 2020).

Type 2 diabetes induction in rats was validated using fasting blood glucose levels measured three days post STZ-NA administration. Rats were considered diabetic if fasting blood glucose levels exceeded 200 mg/dL (PERKENI, 2021; Rudianto, 2011). In the normal control group (K0), fasting glucose levels averaged 72.18 mg/dL, indicating normoglycemia or normal metabolic without glucose regulations. In contrast, STZ-NA-induced groups (K-, K+, P1, and P2) showed significant increases in fasting glucose levels compared to K0, confirming successful diabetes induction. Bonferroni post hoc tests revealed statistically significant differences ($p<0.005$) between K0 and all induced groups. Table 1 below shows a visual representation of GPD values from every group.

Table 1. Fasting Blood Glucose Levels Using STZ-NA Induction

GDP (mg/dl)	Rat Groups					P'
	KO	K-	K+	P1	P2	
After (mean±SD)	72.18 ±0.98[a]	267.57 ±4.98[b]	269.72 ±2.56[b]	271.22 ±3.85[b]	269.18 ±1.88[b]	0.000*

Description: KO : Control group not given STZ-NA induction and not given jeruju leaf brew; K-: group given STZ-NA induction but not given jeruju leaf brew; K+: group given STZ-NA induction and metformin 45mg/kg/day; P1: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 6ml/kg/day; P2: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 12ml/kg/day. P': statistical test One-Way ANOVA, post hoc Bonferroni.

Streptozotocin (STZ), a nitrosourea derivative from *Streptomyces achromogenes* bacteria, is commonly used as a diabetogenic agent. STZ disrupts β-cell function by inhibiting glucose oxidation, suppressing insulin synthesis and secretion, and impairing GLUT-2 transporters. STZ induces β-cell necrosis via DNA alkylation and poly-ADP-ribose polymerase 1 (PARP-1) activation, reducing ATP and increasing oxidative stress (Ghasemi et al., 2014; Meditory & Issn Online, 2021). Nicotinamide (NA), a vitamin B3 derivative, provides antioxidant protection by stabilizing free radicals and nitrogen oxide (NO), inhibiting PARP activation and apoptosis (Ghasemi et al., 2014; Gheibi et al., 2017). In this study, rats were intraperitoneally injected with NA (110 mg/kg) followed 15 minutes later by STZ (45 mg/kg) to model T2DM-like insulin resistance. This model is applied to K-, K+, P1, and P2 to create insulin resistance conditions like pathogenesis T2DM in humans.

One-way ANOVA results indicated significant reductions in TNF-α levels in P1 and P2 groups after 28 days ($p<0.05$). Average TNF-α levels were 7.80 pg/ml (P1) and 6.94 pg/ml (P2), with P2 values approaching those of the standard control (6.03 pg/ml). This indicates that the higher the dose administered, the greater the reduction in TNF-α levels. In contrast, the untreated diabetic group (K-) exhibited elevated TNF-α levels (14.09 pg/ml). This result showed metformin and jeruju leaf brew reduced inflammation reaction, indicating the decreased TNF-a levels in rats induced by STZ-NA compared to those not given intervention. Visual representation from TNF-a is shown Figure 1.

As shown in Figure 1, it is evident that all experimental rats receiving interventions exhibited lower mean levels of TNF-α compared to the negative control group (K-). This finding is attributable to the fact that the K- -group was induced with STZ-NA without receiving any subsequent therapeutic intervention. Meanwhile, the K0 group served as the standard reference, as the rats were in a normal physiological state and were not administered either metformin or jeruju leaf brew.

The HOMA-IR index, calculated from fasting glucose and insulin levels, indicates insulin resistance in T2DM; a higher HOMA-IR value indicates greater insulin resistance (Kaseda et al., 2020). In Figure 2, P1 and P2 interventions significantly reduced insulin resistance ($p<0.05$). P2 achieved a HOMA-IR score of 3.28, lower than P1 (3.75) and K+ (3.74) and approaching normal levels (K0: 2.92). This supports the synergistic effect of metformin and jeruju leaf brew in improving insulin sensitivity. Visual representation from HOMA-IR is shown Figure 2.

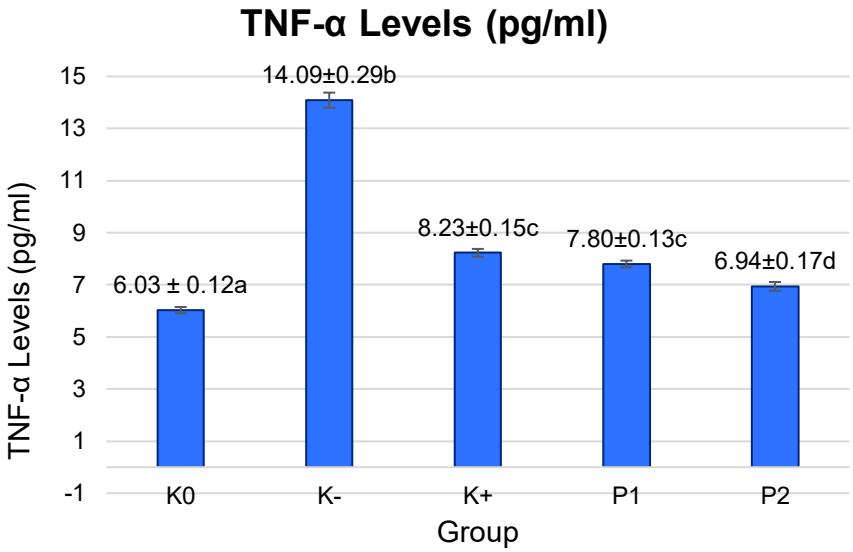

Figure 1. TNF-α Levels After Intervention

Description: KO : Control group not given STZ-NA induction and not given jeruju leaf brew; K-: group given STZ-NA induction but not given jeruju leaf brew; K+: group given STZ-NA induction and metformin 45mg/kg/day; P1: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 6ml/kg/day; P2: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 6ml/kg/day. Statistical test One-Way ANOVA, Alphabetic notation (a,b,c,d): post hoc Bonferroni

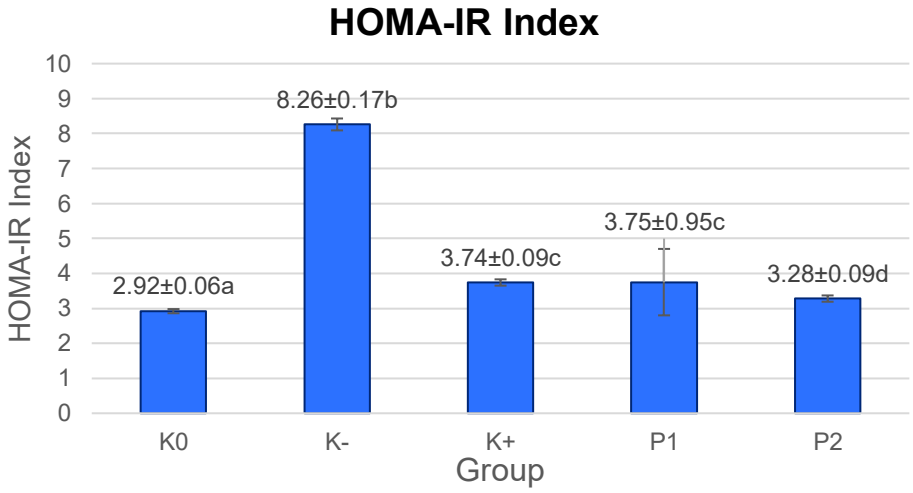

Figure 2. HOMA-IR Index After Intervention

Description: KO : Control group not given STZ-NA induction and not given jeruju leaf brew; K-: group given STZ-NA induction but not given jeruju leaf brew; K+: group given STZ-NA induction and metformin 45mg/kg/day; P1: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 6ml/kg/day; P2: group given STZ-NA induction and given metformin 45mg/kg/day and jeruju leaf brew 6ml/kg/day. Statistical test One-Way ANOVA, Alphabetic notation (a,b,c,d): post hoc Bonferroni

Figure 2 shows that all experimental rats receiving interventions exhibited a lower mean HOMA-IR index than the negative control group (K-). This finding is attributable to the fact that the K- -group was induced with STZ-NA without receiving any subsequent therapeutic intervention. Meanwhile, the K0 group served as the standard reference, as the rats were in a normal physiological state and were not administered either metformin or jeruju leaf brew.

The Bonferroni post hoc analysis observed a statistically significant difference ($p < 0.05$) in the intervention groups treated with jeruju leaf brew. Specifically, type 2 diabetic rats in the P1 and P2 treatment groups, which received both metformin and the herbal brew, showed significantly lower mean TNF-α levels than the positive control group (K+) that received metformin alone, with p-values of 0.005 and 0.000, respectively. Regarding HOMA-IR values, the P1 group had a similar mean value to the K+ group, and no statistically significant difference was found ($p = 1.000$). These results suggest that K+ and P1 interventions were comparably effective in reducing TNF-α and HOMA-IR levels, with only minimal differences. However, the P2 group demonstrated a significantly greater reduction in TNF-α and HOMA-IR levels than the other groups, indicating a superior therapeutic effect.

This result shows metformin intervention and jeruju leaf brew effectively decreased the TNF-α and HOMA-IR result. This may be associated with the jeruju leaf brew, which contains antioxidant compounds such as flavonoids, alkaloids, and saponins. Flavonoids regulate TNF-α synthesis and activation by modulating signaling pathways in producing this pro-inflammatory cytokine. (Margono et al., 2016). TNF-α is a pro-inflammatory cytokine pivotal in the immune response to inflammation, infection, and tissue injury. This cytokine is synthesized by various cells, particularly immune cells such as macrophages, lymphocytes, and dendritic cells. Its presence contributes to the immune response by stimulating the activation of immune cells, increasing vascular permeability, and regulating the migration of immune cells to sites of inflammation. Furthermore, this cytokine regulates the inflammatory process and protects against pathogens and tissue damage (Wardani et al., 2023).

The anti-inflammatory mechanism of flavonoids involves the inhibition of Nuclear Factor Kappa B (NF-kB) activation, which is a major regulator of TNF-α production, thereby supporting the role of flavonoids as anti-inflammatory compounds (Sahoo et al., 2021; Sujono et al., 2021). In situations where there is an increase in the production of reactive oxygen species (ROS) and exposure to pro-inflammatory stimuli, the activation of the NF-kB signaling pathway is triggered. ROS serve as molecular signals that promote the release of NF-kB from its inhibitor complex. This process is initiated by the phosphorylation of IkB-α by IKK (Inhibitor kappa B Kinase), leading to proteasomal degradation of IkB and the release of NF-kB from the cytoplasm. Once unbound, NF-kB translocates to the nucleus, where it binds to DNA and activates the transcription of various target genes, including those encoding pro-inflammatory cytokines such as TNF-α (S.-J. Lee et al., 2015; Peng et al., 2021). Quercetin and fisetin, components of flavonoids, have been shown to inhibit key MAPK components, such as JNK (c-Jun N-terminal Kinase), ERK (Extracellular signal Regulated Kinase), and p38, thereby suppressing NF-kB activation through a reduction in the phosphorylation of IkB and p65. This condition contributes to the reduction in NF-kB accumulation, decreasing TNF-α levels (Zhong et al., 2022; Zu et al., 2021).

This study aligns with previous research indicating that the methanol extract of jeruju leaves significantly inhibits the production of pro-inflammatory cytokines, including TNF-α levels, in lipopolysaccharide-induced peripheral blood mononuclear

cells (Mani Senthil Kumar et al., 2008) Previous studies showed flavonoid on methanolic jeruju leaf extracts at 1.5 mg/kg reduced inflammation by 40% in mice, supporting the anti-inflammatory role of its flavonoids *(Ni Made Sugi Pradnyasuari & Anak Agung Gede Rai Yadnya Putra, 2023).* In another study, jeruju extract nanoemulsions restored gastrointestinal function in ulcer-induced mice after 14 days of intervention. The mechanism of flavonoids in this context involves the enhancement of prostaglandin levels in the gastric mucosa and the inhibition of histamine secretion through the suppression of the enzyme histidine decarboxylase. In addition, flavonoids also contribute to inhibiting cyclooxygenase enzymes, thereby preventing the synthesis of prostaglandins and reducing cellular inflammation (Rizeki et al., 2020).

Glucose metabolism in the body is a complex process tightly regulated by various hormones, with insulin and glucagon as the primary regulators. In the context of insulin resistance, flavonoids act as insulin sensitizers by enhancing IRS-1 and IRS-2 phosphorylation and activating the PI3K/Akt pathway, promoting GLUT-2 and GLUT-4 translocation to the plasma membrane, promoting glucose uptake into muscle and adipose tissues and hepatic glucose suppression (Álvarez-Cilleros et al., 2018; Cordero-Herrera et al., 2013). Alkaloids further enhance insulin receptor responsiveness and glucose uptake, reducing hyperglycemia (Adhikari, 2021).

jeruju leaf brew combined with metformin improved insulin sensitivity and glycemic control. Metformin inhibits hepatic gluconeogenesis and activates AMPK (Martín & Ramos, 2021; Za'abi et al., 2021). Improvement in blood glucose levels may lead to a reduction in serum insulin concentrations, thereby ameliorating the condition of hyperinsulinemia (Deng et al., 2020). These findings suggest that the combination of jeruju leaf brew and metformin synergistically enhances insulin sensitivity and reduces fasting blood glucose levels, potentially offering a more optimal therapeutic outcome than monotherapy. Therefore, jeruju leaf brew holds promise as an adjuvant therapy to support the effectiveness of conventional metformin treatment in the comprehensive management of T2DM. Further studies are needed to determine optimal dosing, safety, and efficacy in human clinical trials.

This study's limitations were that it was carried out at the preclinical stage with experimental animals, so further research is needed to confirm the same effect in humans. Additionally, more tests are required to evaluate the optimal dosage of jeruju leaf brew, its toxicity limits, effectiveness, safety, and mechanism of action in managing T2DM.

CONCLUSION

The jeruju leaf brew (*Acanthus ilicifolius* L) at doses of 6 ml/kgBB/day and 12 ml/kgBB/day significantly reduces TNF-α and HOMA-IR in STZ-NA induced male white rats. Jeruju leaves contain several secondary metabolites, including flavonoids, alkaloids, and saponins, which are considered potential antioxidants, anti-inflammatories, and antidiabetics. This study establishes a foundation for developing herbal remedies, particularly jeruju leaves, as an adjunct therapy to enhance the efficacy of conventional metformin treatment in managing T2DM.

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CONFLICT OF INTEREST

The authors declare no conflict of interest related to this research and its publication.

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