

Jeruju (*Acanthus ilicifolius*) Leaf Infusion Modulates Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) Enzyme Activity in Streptozotocin-Nicotinamide (STZ-NA) Induced Diabetic Rats (*Rattus norvegicus*)**Ade Chairina^{1*}, Diana Nur Afifah¹, Ahmad Syauqy¹**¹ Department of Nutrition Science, Universitas Diponegoro, Semarang, IndonesiaCorresponding Author Email: ade.chairina61@gmail.com

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This work is licensed under a [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License](https://creativecommons.org/licenses/by-nc-sa/4.0/)**ABSTRACT**

Type 2 diabetes mellitus is a metabolic disorder marked by chronic hyperglycemia and oxidative stress, which can reduce the activity of antioxidant enzymes like Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx). *Acanthus ilicifolius* (jeruju) contains antioxidant compounds such as flavonoids and phenolics that may enhance these enzyme activities. Evaluate the effect of *Acanthus ilicifolius* leaf infusion on SOD and GPx activity in Wistar rats induced with type 2 diabetes using Streptozotocin-Nicotinamide (STZ-NA). The experimental design used a post-test only with five groups: normal control (no diabetes, standard feed), positive control (diabetes + metformin 45 mg/kgBW), negative control (diabetes without treatment), treatment group 1 (diabetes + metformin 45 mg/kgBW + jeruju infusion 1.2 ml/200gBW), and treatment group 2 (diabetes + metformin 45 mg/kgBW + jeruju infusion 2.4 ml/200gBW). Treatments were given daily for 30 days, and all rats had free access to food and water. At the end of the study, blood samples were collected to measure SOD and GPx activity using spectrophotometry. The combination of *Acanthus ilicifolius* infusion and metformin particularly at the 2.4 ml/200gBW dose, was associated with elevated SOD P2 treatment group (76.23 ± 3.65 U/mL; $p = 0.000$ (<0.05)) and GPx activities P2 (52.11 ± 0.76 U/mL; $p = 0.000$ (<0.05)) compared to the untreated diabetic group K- (SOD) (28.43 ± 3.17 U/mL) and K- (GPx) (24.18 ± 1.08 U/mL). These findings proves *Acanthus ilicifolius* infusion holds potential as an adjuvant therapy to mitigate oxidative stress in type 2 diabetes by enhancing SOD and GPx enzyme activities.

Key Messages:

- Jeruju leaf infusion reduced diabetes-related weight loss and significantly increased SOD and GPx antioxidant enzyme activity in rats.
- The effects were dose-dependent and greater at 2.4 ml.

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GRAPHICAL ABSTRACT

Exploring Jeruju's Impact on Diabetes



INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disease that poses a global health challenge with an increasing prevalence in both developed and developing countries. The World Health Organization (WHO) predicts that the number of diabetes cases will reach 366 million by 2030(1), while the International Diabetes Federation (IDF) estimates that the prevalence of diabetes in Indonesia will rise to 12.2% by 2045(2). Among all DM cases, approximately 90% are type 2 diabetes mellitus (T2DM), characterized by insulin resistance and chronic hyperglycemia(3).

Hyperglycemia in T2DM contributes to increased production of Reactive Oxygen Species (ROS), particularly superoxide radicals in endothelial cells of the mitochondria. In addition to the activation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase, chronic hyperglycemia also enhances ROS production via multiple metabolic pathways, including the polyol pathway, increased formation of advanced glycation end-products (AGEs), and activation of protein kinase C (PKC). These pathways amplify oxidative stress and are implicated in the development of diabetic complications (4). These superoxide radicals are produced as a byproduct in small amounts by Nicotinamide Adenine Dinucleotide Phosphate (NADPH)(5). To counteract oxidative stress, the body activates its endogenous antioxidant defense system through enzymes such as Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx)(6). SOD functions to convert superoxide radicals into hydrogen peroxide, while GPx detoxifies hydrogen peroxide and lipid peroxides(7). However, excessive ROS production can lead to the inactivation of these antioxidant enzymes, exacerbating complications associated with T2DM(8).

One approach to reducing ROS production and enhancing antioxidant enzyme activity is through the administration of exogenous antioxidants(9). Exogenous antioxidants can be obtained from various natural sources, including fruits, vegetables, herbal beverages, and cereals(10). One bioactive compound with significant potential in reducing the risk of oxidative stress-related diseases is flavonoids. Flavonoids have been extensively studied for their antioxidant activity, which can improve insulin sensitivity and suppress excessive ROS production(11). Flavonoids, a diverse group of plant polyphenols, play a significant role in mitigating oxidative stress and its associated damage. These compounds possess potent antioxidant properties, enabling them to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby reducing oxidative stress. Flavonoids achieve this by modulating various intracellular signaling pathways, including the Nrf2 antioxidant response and NF- κ B pathways, which are crucial for cellular defense mechanisms(11,12). Additionally, flavonoids inhibit the activity of ROS-generating enzymes such as cyclooxygenase (COX), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS), further contributing to their anti-inflammatory effects(14).

Mangrove plants are one of the natural sources rich in flavonoids, steroids, terpenes, and alkaloids(15). Several studies have shown that mangrove extracts possess broad pharmacological activities, including anti-diabetic effects(16). One mangrove species with significant health potential is the jeruju plant (*Acanthus ilicifolius*). This plant is commonly found in coastal areas, riverbanks, and regions with muddy or brackish water. In Kebumen Regency, Central Java, jeruju leaves have traditionally been used as a herbal drink believed to benefit individuals with diabetes. Phytochemical analysis has revealed that this plant contains 8.4 mg/10 g of flavonoids and 17.22 mg/10 g of phenolics, which play a crucial role in antioxidant activity(17). Previous studies have also identified specific bioactive compounds in *A. ilicifolius*, such as glycosides, saponins, sterols, terpenoids, alkaloids which are known for their strong antioxidant and free radical-scavenging activities(18).

In addition to its traditional medicinal uses, jeruju leaves have been reported to exhibit various pharmacological activities, including anti-inflammatory, antimicrobial, anticancer, hepatoprotective, and osteoblastic effects(19). However, further investigation of jeruju leaves as an antioxidant agent in the context of T2DM remains limited. Therefore, this study aims to evaluate the effect of jeruju leaf (*Acanthus ilicifolius*) infusion at varying doses on the activity of SOD and GPx enzymes in streptozotocin-nicotinamide (STZ-NA)-induced white rats.

METHODS

This study utilised an *experimental post-test only design with multiple groups*. Male Wistar rats (*Rattus norvegicus*) aged 8 weeks and weighing 150-200 grams were used as experimental subjects. The rats were randomly assigned to five groups (n=6) using simple randomisation, generated with Microsoft Excel to ensure an equal and unbiased distribution. The sample size was determined using the Federer formula, which is commonly applied in preliminary animal studies to ensure minimum statistical requirements are met.

Male Wistar rats were randomly assigned into five groups: a normal control group that received no diabetes induction and was given only standard feed; a positive control group that was induced with diabetes and treated with metformin at a dose of 45 mg/kg body weight (BW) orally per day; a negative control group that was induced with diabetes but received no additional treatment apart from standard feed; a treatment group 1 that was induced with diabetes and given *Acanthus ilicifolius* leaf infusion at a dose of 1.2 ml per 200 g BW; and a treatment group 2 that was induced with diabetes and administered the infusion at a dose of 2.4 ml per 200 g BW. Diabetes was induced using a combination of Streptozotocin (STZ) and Nicotinamide (NA). All animals were provided food and water ad libitum throughout the study. The treatment was administered daily for 30 days. At the end of the treatment period, blood samples were collected, and the activities of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) enzymes were quantitatively measured using spectrophotometric methods, based on the catalytic action of each enzyme on its specific substrate.

The diabetes model was induced by intraperitoneal injection of Nicotinamide (110 mg/kgBW) followed by Streptozotocin (45 mg/kgBW). The STZ-NA model is a widely used experimental approach to induce Type 2 Diabetes Mellitus (T2DM) in laboratory animals. This model leverages the diabetogenic properties of streptozotocin (STZ) combined with the protective effects of nicotinamide (NA) to partially safeguard pancreatic β -cells, thereby mimicking the pathophysiology of T2DM(20). Rats were considered diabetic if fasting blood glucose levels were ≥ 250 mg/dL after 72 hours. The jeruju leaf infusion was prepared by brewing 2 g of dried *Acanthus ilicifolius* leaves in 100 ml of hot water (95°C) for 15 minutes, the infusion was administered via oral gavage once daily for 28 days at two dose volumes: 1.2 ml/200 g BW and 2.4 ml/200 g BW. The doses of 1.2 ml/200 g BW and 2.4 ml/200 g BW for *Acanthus ilicifolius* leaf infusion were adapted from Mahammad et al., who administered 6 ml/kg BW of herbal tea infusion in diabetic rats. The preparation involved infusing 2 g of jeruju leaves in 100 ml of 90°C water for 10 minutes. The infusion was given once daily via oral gavage as an adjuvant to metformin (9 mg/200 g BW) (21).

SOD and GPx enzyme activities were analyzed from serum samples collected through retro-orbital plexus blood sampling. The activities of SOD and GPx were measured using rat-specific commercial ELISA kits (Elabscience®, USA) according to the manufacturer's instructions. Absorbance was read at 450 nm

using a microplate reader. The type of SOD measured was total SOD activity. Standard curves were used to calculate enzyme concentrations in the sample. The preparation of jeruju leaf infusion began with the harvesting of leaves from the first to third shoots using scissors, due to the presence of thorns. The harvested leaves were cleaned by removing the thorns and separating them from the midribs, then sliced thinly. The sliced leaves were dried using a food dehydrator at 80°C for 150 minutes. Once dried, the leaves were ground using a blender to obtain a coarse powder (22). The enzymatic activities were measured using a spectrophotometric method at 450 nm wavelength. The mean and standard deviation of all the collected data were reported using SPSS version 26 for Windows (IBM Analytics, Armonk, NY, USA). Statistical tests were carried out to determine the differences before and after the intervention in BW. In addition, the paired t-test was adopted for the BW before and after intervention. One-way ANOVA statistical test, followed by posthoc Bonferroni with a significant value of $p < 0.05$ was carried out to determine the difference of fasting blood glucose (FBG), superoxide dismutase (SOD), and glutathione peroxidase (GPx) among groups. In addition, the adopted Pearson correlation test was performed for the relationship between SOD and GPx.

This study has received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University, with approval number No. 089/EC-H/KEPK/FK-UNDIP/VIII/2024, dated Agustus 29, 2024. It adheres to international guidelines for the care and use of laboratory animals. The experimental protocol is illustrated in Figure 1.

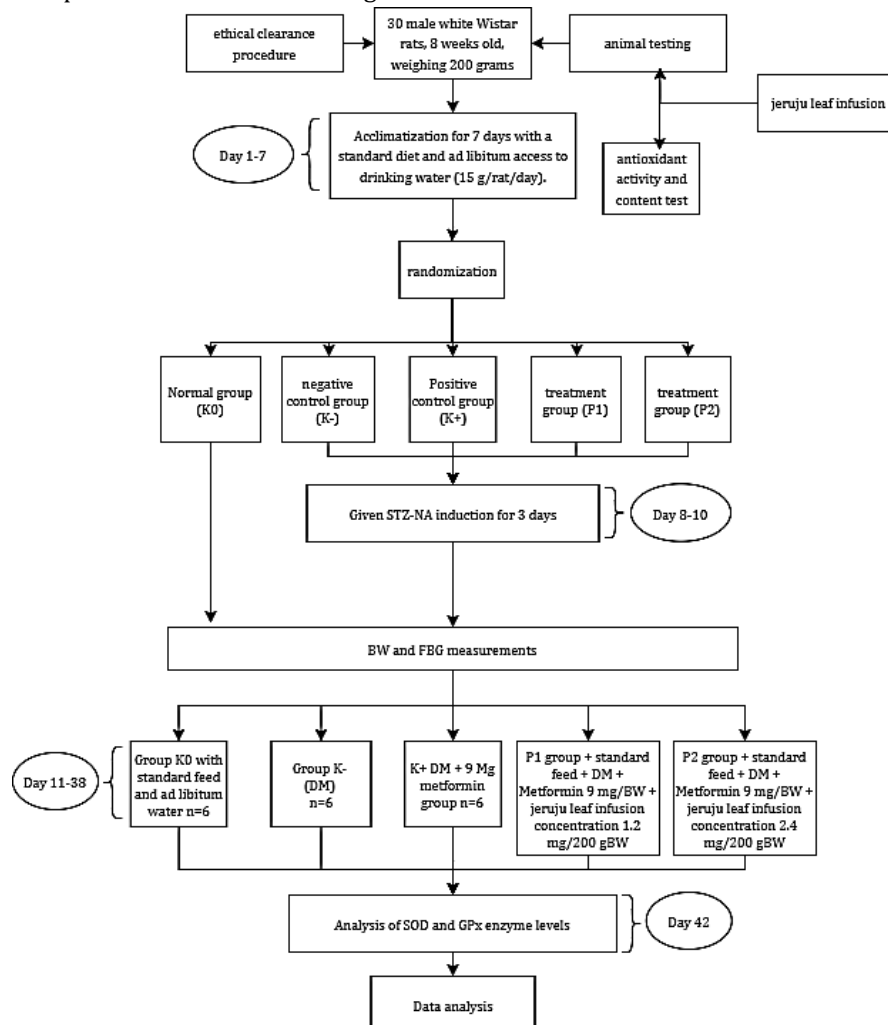


Figure 1. Flowchart of the experimental design showing the grouping, STZ-NA induction, treatment administration, and analysis of SOD and GPx enzyme activity in diabetic Wistar rats.

BW = Body Weight; FBG = Fasting Blood Glucose; STZ-NA = Streptozotocin-Nicotinamide; DM = Diabetes Mellitus; K = Normal control group; K- = Negative control group (DM without treatment); K+ = Positive control group (DM + metformin); P1 = Treatment group (DM + metformin + *Acanthus ilicifolius* infusion at 1.2 mg/200 g BW); P2 = Treatment group (DM + metformin + *Acanthus ilicifolius* infusion at 2.4 mg/200 g BW); *ad libitum* = free access to food and/or water; mg/BW = milligram per body weight.

RESULTS

The results showed that Jeruju leaf infusion significantly increased BW, SOD and GPx activity seen in Figure 2. Jeruju leaf infusion was able to increase SOD and GPx activity in type 2 diabetes mellitus rats (Table 1). According to Nguyen et al. (2021), the optimum brewing time was 90°C for 40 minutes this time will produce flavonoid content 60,8% in black tea(23). In this study, the infusion was prepared by brewing 2 g of dried *Acanthus ilicifolius* leaves in 100 ml of hot water (90°C) for 10 minutes. The resulting infusion had a flavonoid content of 50.112 mgQE/g based on analysis of jeruju leaf brewing, suggesting a high concentration of bioactive compounds that may contribute to its antioxidant properties.

Table 1. Initial Characteristics and Daily Feed Intake

Group	Initial body weight (g)	Initial FBG (mg/dL)	Daily feed intake (g/rat)	Intervention intake (ml/rat)	Number of surviving rats
Normal control (K)	195.50 ± 2.88	72.18 ± 0.98	19.55 g/day	-	6
Negative control (K-)	179.00 ± 2.36	267.56 ± 4.98	17.9 g/day	-	6
Positive control (K+)	180.33 ± 2.16	269.72 ± 2.56	18 g/day	9 mg metformin	6
Treatment 1 (P1)	180.83 ± 2.63	271.22 ± 3.85	18.08 g/day	9 mg metformin + 1.2 ml jeruju infusion	6
Treatment 2 (P2)	180.66 ± 2.16	269.18 ± 1.88	18.06 g/day	9 mg metformin + 2.4 ml jeruju infusion	6

Body weight

Group K was not given STZ-NA induction, while the other 4 groups received the induction. There were changes in the body weight and the greatest decrease in the STZ-NA induced group was the K+ group. K showed the smallest increase in body weight because the group was not induced by STZ-NA. Administration of STZ causes weight loss, increased blood glucose, and decreased insulin levels(24). The weight loss in the intervention group was lower when compared to the control group, Specifically, the intervention group (STZ + NA) exhibited a lower weight loss compared to the control group, indicating a protective effect against weight loss in the context of type 2 diabetes mellitus (T2DM)(24). In addition, the standard deviation value was relatively large compared to the results. Groups K-, K+, P1, and P2 showed a higher decrease in body weight compared to K, with an average body weight of 188,47 g in each group given STZ-NA. On the 3rd day, the weight change of the control and STZ-NA groups was 195,5 g and 179 g, respectively. A statistical test with one-way ANOVA showed no significant difference in the pre-STZ-NA group; hence, the sample had been randomized successfully.

Caloric restriction.

The K-, K+, P1, and P2 groups were given a normal diet during the 4 weeks of the intervention period. In addition, the rats were given intervention in the form of Metformin or Jeruju.

SOD and GPx.

SOD and GPx both of antioxidant enzyme play a role in the body's antioxidant defense system and are often used to evaluate the level of oxidative damage and the effectiveness of therapeutic interventions in managing diabetes(25). The value after jeruju brewing intervention is shown in Figure 2B and there was a significant difference in the mean between groups ($p < 0,05$), SOD and GPx values were affected by intervention duration and doses. SOD and GPx values of the intervention group P1 and P2 were significantly different from K-. In addition, SOD in metformin group and P1 were not significantly different was provided the same improvement effect as the metformin group (K+). Therefore, 2.4 ml jeruju brewing had a better effect than metformin on SOD and GPx value in diabetes mellitus. In the intervention group, P2 had the increased SOD and GPx value, followed by P1. There were significant differences between

groups P1 and P2, hence the effect was dose-dependent. As the best dose, P2 increased SOD by 76,23% and GPx value by 52,11% compared to the negative control

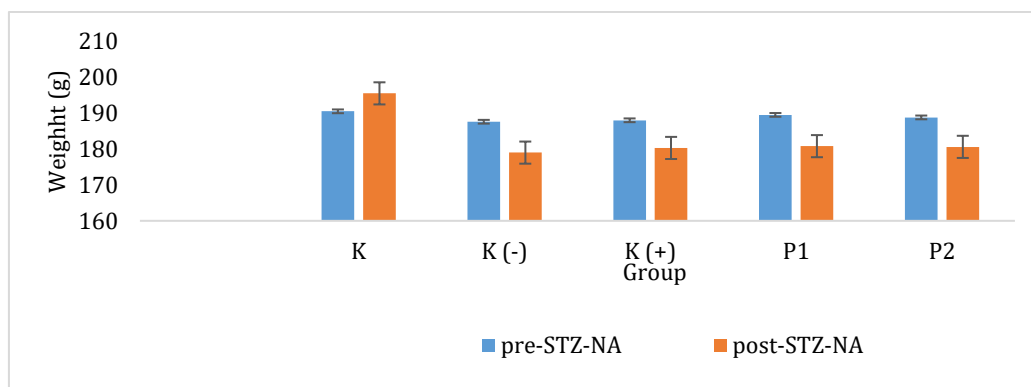


Figure 2. Mean body weight of rats in each experimental group before and after STZ-NA induction. Data are presented as mean \pm SD. Groups include Normal Control (K), Diabetic Control (K-), Metformin-treated (K+), and Jeruju infusion with metformin-treated groups (P1 and P2). Body weights were measured prior to and 72 hours after induction with streptozotocin-nicotinamide (STZ-NA).

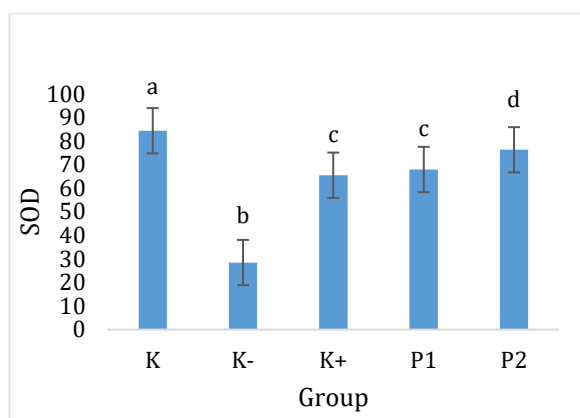


Figure 3. SOD diagram

P: Treatment; K+: Metformin; K-: Diabetic group; K: Normal control; Different superscript letters (a,b) indicate significant differences between groups ($p < 0.001$).

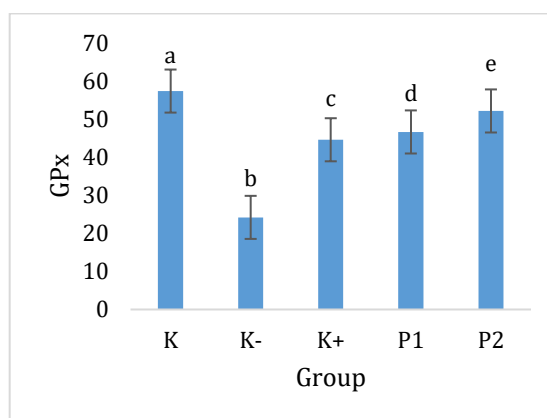


Figure 4. GPx diagram

P: Treatment; K+: Metformin; K-: Diabetic group; K: Normal control; Different superscript letters (a,b) indicate significant differences between groups ($p < 0.001$).

DISCUSSION

Body weight

The initial physiological parameters, particularly body weight and feed intake, reflect a well-executed randomization of experimental animals. The diabetic groups (K-, K+, P1, and P2), all induced using streptozotocin-nicotinamide (STZ-NA), displayed comparable initial body weights (179.00 ± 2.36 g to 180.83 ± 2.63 g), suggesting that the β -cell toxicity induced by STZ, followed by partial protection from nicotinamide, did not cause systemic cachexia or acute metabolic disturbances at baseline. The slightly higher body weight observed in the non-diabetic control group (195.50 ± 2.88 g) is physiologically consistent with intact insulin signaling and glucose utilization, which are crucial for anabolic processes such as adipogenesis and protein synthesis.

Uniformity in daily feed intake (17.9 – 19.55 g/day) across all groups further supports the notion that neither the diabetic induction nor the interventions impaired central appetite regulation, primarily governed by hypothalamic neuropeptides like NPY and POMC or peripheral hunger signals like ghrelin or leptin(26). The absence of mortality in all groups signifies not only the tolerability of metformin but also suggests that the phytochemical components of jeruju infusion, likely including flavonoids, polyphenols, and other antioxidant compounds, do not exert acute cytotoxicity at doses of 1.2 ml and 2.4 ml per rat per day. These phytoconstituents are hypothesized to modulate cellular redox homeostasis by scavenging reactive oxygen species (ROS) and potentially upregulating endogenous antioxidant pathways such as the

Nrf2-ARE signaling axis(27). Their inclusion alongside metformin, which itself activates AMP-activated protein kinase (AMPK) to restore energy balance and suppress hepatic gluconeogenesis, provides a rational combinatorial approach for metabolic disease modulation(28).

SOD activity

The present study demonstrates significant modulation of Superoxide Dismutase (SOD) activity across experimental groups ($p < 0.001$), reflecting the oxidative status and the impact of interventions on endogenous antioxidant defenses at the molecular level. In the normal control group (K), SOD activity was highest (84.31 ± 3.17 U/mL), indicating a well-maintained redox homeostasis under physiological conditions. SOD, an essential first-line defense antioxidant enzyme, catalyzes the dismutation of superoxide anion radicals ($O_2^{\bullet-}$) into hydrogen peroxide (H_2O_2), thereby reducing cellular oxidative burden and preventing the propagation of lipid peroxidation and DNA damage(6).

Conversely, the negative control group (K-) exhibited a profound suppression of SOD activity (28.43 ± 3.17 U/mL), consistent with the oxidative damage typically induced by streptozotocin (STZ)(29). STZ enters pancreatic β -cells via the GLUT2 transporter and causes DNA alkylation, which triggers excessive ROS production, NAD⁺ depletion, and mitochondrial dysfunction(30). NA partially protects β -cells, yet the imbalance in redox state persists, as reflected by SOD suppression.(31) This SOD depletion compromises cellular antioxidant defense, enhancing the susceptibility of tissues to oxidative damage associated with hyperglycemia(32).

Metformin treatment (K+) restored SOD activity significantly (65.44 ± 2.75 U/mL), underscoring its pleiotropic role beyond glucose lowering. Metformin activates AMP-activated protein kinase (AMPK), a key energy-sensing molecule that promotes antioxidant gene expression, partly via Nrf2 (nuclear factor erythroid 2-related factor 2) pathway activation(33). Nrf2 translocates to the nucleus under oxidative stress and binds to the antioxidant response element (ARE), promoting transcription of antioxidant enzymes including SOD1 and SOD2(34).

The combination therapies in P1 (1.2 ml jeruju + metformin) and P2 (2.4 ml jeruju + metformin) further enhanced SOD activity (67.89 ± 2.16 and 76.23 ± 3.65 U/mL, respectively), with P2 approaching near-normal levels, indicating a dose-dependent augmentation of antioxidant capacity. Jeruju (*Acanthus ilicifolius*) leaf infusion likely contributes to this effect through its rich content of polyphenols and flavonoids such as apigenin, luteolin, and caffeic acid derivatives, which are known to directly scavenge ROS(35). The enhanced SOD activity in P2 proves a potential synergistic interaction between metformin and jeruju constituents in restoring redox balance. Moreover, this points toward epigenetic modulation of antioxidant defense mechanisms. Overall, the findings underscore the capacity of jeruju leaf infusion to ameliorate oxidative stress at the molecular level. When combined with metformin, the plant-based intervention may offer a multitargeted therapeutic approach in mitigating oxidative complications of type 2 diabetes.

GPx activity

The current study evaluated the impact of *Acanthus ilicifolius* (jeruju) leaf infusion, both alone and in combination with metformin, on Glutathione Peroxidase (GPx) activity in streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats. The results demonstrate significant variations in GPx activity across different treatment groups ($p < 0.001$), highlighting the potential antioxidative benefits of the interventions. The negative control group (K-), representing untreated diabetic rats, exhibited a marked reduction in GPx activity (24.18 ± 1.08 U/mL) compared to the normal control group (K) (57.33 ± 1.21 U/mL). The reduction of glutathione peroxidase (GPx) activity in the streptozotocin-nicotinamide (STZ-NA)-induced diabetic group proves that STZ-NA leads to oxidative stress, which decreases the body's antioxidant defense mechanisms(36). Treatment with metformin alone (K+) resulted in a significant improvement in GPx activity (44.55 ± 0.74 U/mL), higher than K- group. These results are in line with previous studies showing that metformin reduces oxidative stress through activation of the AMP-activated protein kinase (AMPK) pathway(37).

Notably, the groups receiving combined treatments of metformin and jeruju leaf infusion (P1 and

P2) showed further enhancements in GPx activity. Group P1 (metformin + 1.2 ml jeruju) exhibited a GPx activity of 46.60 ± 1.01 U/mL, whereas group P2 (metformin + 2.4 ml jeruju) demonstrated an activity of 52.11 ± 0.76 U/mL. The superior GPx activity in P2 proves a dose-dependent synergistic effect between metformin and the bioactive compounds in jeruju, such as flavonoids and phenolics, known for their antioxidative properties (38).

These findings indicate that incorporating jeruju leaf infusion as an adjuvant therapy alongside metformin may bolster the antioxidant defense system in diabetic conditions, potentially mitigating oxidative stress-related complications. However, further studies are warranted to elucidate the underlying mechanisms and to assess the clinical relevance of these results.

In this study, SOD and GPx activities were only measured at the end of the treatment. Pre-treatment measurements were not performed due to ethical and technical considerations, as repeated sampling in small laboratory animals may induce stress and influence experimental outcomes. To minimize variability, randomization was conducted after diabetic induction, and all animals were maintained under identical conditions. However, we acknowledge that the lack of baseline enzyme levels limits the ability to fully confirm homogeneity across groups prior to treatment initiation.

CONCLUSION

The administration of Jeruju (*Acanthus ilicifolius*) leaf infusion significantly enhances the activity of Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) in STZ-NA-induced diabetic rats, which plays a critical role in the progression of type 2 diabetes mellitus (T2DM). The 2.4 ml/200gBW dose showed greater efficacy than the 1.2 ml/200gBW dose, indicating a dose-dependent effect. Further research is needed to explore the long-term effects and underlying molecular mechanisms of Jeruju in diabetes management.

One limitation of this study is the absence of pre-treatment measurements of SOD and GPx activity, which may have provided a more accurate assessment of the intervention's effects. Although standard randomization and housing protocols were followed, potential baseline differences in antioxidant enzyme levels cannot be entirely ruled out. Further research is warranted to identify the specific bioactive compounds responsible for the antioxidant activity, elucidate their molecular mechanisms (e.g., Nrf2 activation, NOX inhibition), and assess the long-term efficacy and safety of Jeruju infusion in chronic diabetes management.

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CONFLICTS OF INTEREST

The author has no conflict of interest.

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