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Antihyperglycemia Effectiveness of The Combination of Ethanol Extract of Moringa Leaves (*Moringa oleifera* L.) and Paliasa Leaves (*Kleinhovia hospita* L.) in Male Mice (Mus Musculus) Induced by Alloxan

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ABSTRACT

Introduction*: Hyperglycemia is a condition characterized by increased fasting blood glucose levels ≥126 mg/dL or oral glucose tolerance test ≥200 mg/dL. Herbal medicine is explored as an alternative treatment, with Moringa leaf extracts (MLE) and Paliasa leaf extracts (PLE) showing potential as antihyperglycemic agents.* ***Aims****: This study aims to standardize these extracts to ensure consistent quality for traditional medicine use and to assess their antihyperglycemic effects when combined.* ***Methods****: This study is an experimental-laboratory research focused on quality standardization of the extracts. Both specific and non-specific parameters are tested. The antihyperglycemic effects are evaluated using alloxan-induced hyperglycemic mice, divided into eight groups: negative control (0.5% Na.CMC), positive control (glibenclamide 5 mg/kg BW), single extract groups (MLE at 0.63 mg/kg BW and PLE at 1.05 mg/kg BW), and combination MLE:PLE groups with various doses (0.63:1.05 mg/kg BW, 0.315:1.05 mg/kg BW, 0.63:0.525 mg/kg BW, and 0.315:0.525 mg/kg BW). The treatment is administered for eight days, and data is analyzed using One-Way ANOVA and post hoc Tukey tests (significance at p<0.05).* ***Results****: Both extracts meet the quality standardization criteria. The extracts show significant antihyperglycemic effects both individually and in combination MLE:PLE at various dose (0.63:1.05 mg/kg BW, 0.315:0.525 mg/kg BW, 0.63:0.525 mg/kg BW, and 0.315:1.05 mg/kg BW).* ***Conclusion****: Both single and combination MLE and PLE meet quality standards and effective antihyperglycemic activity. These findings suggest their potential use as traditional herbal medicine for managing hyperglycemia.*

KEYWORDS*: Hyperglycemia, moringa, paliasa, extract*

INTRODUCTION

Hyperglycemia is a medical condition characterized by elevated blood glucose levels exceeding the normal physiological range and serves as a hallmark of diabetes mellitus (DM) (PERKENI, 2015). DM is a chronic metabolic disorder marked by persistent hyperglycemia due to defects in insulin secretion, insulin action, or both. It is classified into three main types: type 1, type 2, and gestational diabetes (Ministry of Health RI, 2020). According to the

International Diabetes Federation (IDF), approximately 463 million individuals aged 20–79 years worldwide were affected by diabetes in 2019, corresponding to a prevalence rate of 9.3%. This prevalence increases significantly to 19.9% among individuals aged 65–79 years (Ministry of Health of the Republic of Indonesia, 2020). In Indonesia, diabetes ranks fifth among the top ten diseases in Southeast Sulawesi, with 13,946 reported cases (Provincial Health Office of Southeast Sulawesi, 2022).

The World Health Organization (WHO) advocates the use of traditional medicines derived from natural sources due to their advantages over synthetic drugs, particularly in minimizing adverse effects commonly associated with chemical medications (Latief et al., 2021; Priyoherianto et al., 2021). Numerous medicinal plants have been identified to contain bioactive compounds with potent antihyperglycemic properties. Among them, *Moringa oleifera* L. (moringa leaves) and *Kleinhovia hospita* L. (paliasa leaves) have traditionally been utilized for their antidiabetic, anti-inflammatory, antibacterial, and antioxidant activities (Susanty, Ridnugrah, & Chaerrudin, 2019).

Moringa leaves are rich in flavonoids, tannins, steroids, terpenoids, saponins, and phenolic compounds, which exhibit antioxidant properties and inhibit the α-glucosidase enzyme, thereby reducing glucose absorption in the intestines (Bhattacharya et al., 2018). On the other hand, paliasa leaves contain saponins, cardenolides, bufadienolides, anthraquinones, and triterpenoids, which can stimulate insulin secretion from pancreatic β-cells, subsequently lowering blood glucose levels (Rachmatiah, T., et al. 2015; Shehadeh et al., 2021; Liu et al., 2015). The combination of these two plants has shown a synergistic effect that enhances their overall antihyperglycemic efficacy (Septiawan et al., 2020).

The antihyperglycemic mechanisms of these plants involve multiple biochemical pathways. Flavonoids in moringa leaves reduce aldose reductase activity, promote pancreatic cell regeneration, increase insulin secretion, and inhibit α-glucosidase enzyme activity, thereby decreasing glucose absorption (Bhattacharya et al., 2018). Meanwhile, terpenoids in paliasa leaves activate AMP-activated protein kinase (AMPK), which regulates glucose translocation, enhances peripheral glucose metabolism, and stimulates insulin release (Lau et al., 2015). The inhibition of α-glucosidase by these phytochemicals resembles the mechanism of action of acarbose, a pharmaceutical agent used to reduce postprandial hyperglycemia.

Experimental studies have demonstrated that ethanol extracts of paliasa leaves reduce blood glucose levels in

alloxan-induced hyperglycemic mice in a dose-dependent manner, with an optimal dose of 750 mg/kg body weight. Similarly, ethanol extracts of moringa leaves at a dose of 300 mg/kg body weight significantly decreased fasting blood glucose levels and improved insulin resistance in diabetic rats (Yuliana et al., 2013; Chinedu et al., 2015; Azizah et al., 2018). Alloxan is widely employed to induce hyperglycemia in male mice, as it selectively destroys pancreatic β-cells, mimicking type 1 diabetes mellitus. Male mice are preferred in such studies due to their higher sensitivity to alloxan and the absence of hormonal fluctuations that may affect experimental outcomes, which are commonly observed in female mice.

The integration of moringa and paliasa leaves as natural antihyperglycemic agents demonstrates promising therapeutic potential. The combination of medicinal plants is commonly employed in herbal therapy to enhance efficacy through synergistic effects, as reported in antihyperglycemic studies by Mahmudah et al. (2025). Therefore, this study aims to further elucidate the combined antihyperglycemic effects of moringa and paliasa leaves and explore their underlying mechanisms in hyperglycemia management, thereby supporting the development of safer and more effective antidiabetic therapies.

METHODS

Sample Collection

The samples of *Moringa oleifera* L**.** leaves and *Kleinhovia hospita* L. leaves used in this study were collected from West Muna Regency, Southeast Sulawesi, Indonesia. The collected plant samples were identified at the Pharmacy Laboratory, Mandal Waluya University, Kendari, to confirm the correct species used in the study. This identification step aimed to avoid errors in raw material collection and to differentiate between similar plant species.

Sample Preparation

Moringa oleifera and *Kleinhovia hospita* leaves were collected from the first to at least the seventh leaf stalk, avoiding yellowing leaves to ensure optimal quality. The leaves underwent wet sorting to remove dirt and foreign materials, then washed with clean water, chopped into smaller pieces, and dried in the shade to reduce moisture content and inhibit microbial growth. After dry sorting to eliminate any remaining impurities, the dried leaves (simplicia) were stored separately.

The dried Moringa leaves (500 g) were extracted using the maceration method with 96% ethanol at a 300 g:2.25 mL ratio for approximately three days until saturation. The mixture was filtered, and

the residue was remacerated with the same solvent. The combined filtrates were concentrated using a rotary evaporator. Similarly, *Kleinhovia hospita* leaves were macerated in 96% ethanol for 3×24 hours with occasional stirring. The mixture was filtered, and the filtrate was evaporated using a vacuum rotary evaporator to obtain a thick extract.

Preparation of Na.CMC Suspension, Glibenclamide Suspension, and Alloxan Solution

A 0.5% Na.CMC suspension was prepared by dissolving 0.5 g of Na.CMC in distilled water preheated to 70°C, and the volume was adjusted to 100 mL. The solution was stirred until completely homogeneous. Glibenclamide was prepared as a positive control by converting the human dose (5 mg) to a mouse equivalent dose using the formula 0.0026 × 5 mg = 0.013 mg/20 g body weight, or 0.006 mg/g body weight. The required dose was dissolved in the prepared 0.5% Na.CMC suspension and administered orally at a maximum volume of 1 mL.

Alloxan monohydrate was used to induce hyperglycemia. A total of 150 mg of alloxan was dissolved in 0.9% NaCl and the volume was adjusted to 25 mL in a volumetric flask, resulting in a 6 mg/mL solution. Each mouse (±20 g) received 3.5 mg of alloxan via intraperitoneal injection.

Preparation of Test Animals

The test animals used were healthy male mice (*Mus musculus*), weighing between 20 and 30 grams. A total of 40 mice were divided into 8 treatment groups, each containing 5 mice. Prior to the experiment, all mice were acclimatized for 7 days under identical conditions and fasted for approximately 8 hours before treatments.

Phytochemical Screening

Phytochemical screening was conducted on the concentrated extracts to identify the presence of flavonoids, tannins, saponins, alkaloids, steroids, and triterpenoids.

Alkaloid test

A total of 0.5 g of the extract was mixed with 1 mL of 2N HCl and 9 mL of distilled water, heated in a water bath for 2 minutes, cooled, and filtered. Three test tubes were each filled with 0.5 mL of the filtrate and treated with Mayer's, Bouchardat's, and Dragendorff's reagents, respectively. The presence of alkaloids was indicated by turbidity or precipitate in at least 2 of the 3 tests (Ministry of Health RI, 1995).

Flavonoid test

A total of 10 g of the sample was boiled with 10 mL of hot water for 5 minutes and filtered while hot. To 5 mL of the filtrate, 0.1 g of magnesium powder, 1 mL of concentrated HCl, and 2 mL of amyl alcohol were added. A red, yellow, or orange color in the amyl alcohol layer indicated the

presence of flavonoids (Ministry of Health RI, 1995).

Saponin test

A 0.5 g sample was added to 10 mL of hot water, cooled, and shaken vigorously for 10 seconds. The formation of stable foam (1–10 cm) that persisted for at least 10 minutes and was unaffected by the addition of 1 drop of 2N HCl indicated the presence of saponins (Ministry of Health RI, 1995).

Tannin test

A total of 1 g of the sample was boiled with 100 mL of distilled water for 3 minutes, cooled, and filtered. Then, 2 mL of the filtrate was mixed with 1–2 drops of 1% $FeCl_3$ solution. A blue-black or green-black color indicated the presence of tannins (Ministry of Health RI, 1995).

Steroid and triterpenoid test

A total of 1 g of the sample was macerated with 20 mL of *n*-hexane for 2 hours and filtered. The filtrate was evaporated, and Liebermann–Burchard reagent was added to the residue. A blue or blue-green color indicated the presence of steroids, while a red, pink, or purple color indicated triterpenoids (Harborne, 1987).

Specific Parameters

Extract identity

The identity parameter aims to provide objective identification of the plant source, including the extract name, Latin name, and local (Indonesian) name (Ministry of Health RI, 2000).

Organoleptic test

Organoleptic evaluation was conducted to observe the physical characteristics of the extract, including form, color, odor, and taste (Ministry of Health RI, 2000).

Water-soluble extract content

A total of 5 g of extract was macerated in 250 mL volumetric flask with 100 mL of chloroform-saturated water for 24 hours. The mixture was shaken repeatedly for the first 6 hours and allowed to stand for the next 18 hours. After filtration, 20 mL of the filtrate was evaporated to dryness in a porcelain dish at 105°C and weighed until a constant weight was obtained. Water-soluble extract content (%) was calculated as:

$$\frac{\text{Weight of extract residue}}{\text{Weight of sample}} \times 100$$

Ethanol-soluble extract content

A total of 5 g of extract was macerated in 250 mL volumetric flask with 100 mL of 96% ethanol for 24 hours. The mixture was shaken for the first 6 hours and left to stand for 18 hours. It was filtered quickly to avoid ethanol evaporation. 20 mL of the filtrate was evaporated to dryness at 105°C and weighed until constant weight was reached. Ethanol-soluble extract content (%) was calculated as:

$$\frac{\text{Weight of extract residue}}{\text{Weight of sample}} \times 100$$

Non-Specific Parameters

Loss on drying

An evaporating dish was heated at 105°C for 30 minutes and weighed until the difference between two consecutive weighings was no more than 0.005 g. Then, 2 g of the sample was placed in the dish and dried at 105°C for 5 hours. Drying continued in 10-minute intervals until the difference between weighings did not exceed 0.25% (Ministry of Health RI, 2000). Loss on drying (%) was calculated as: $\frac{(a-b)}{a} \times 100$

a = initial weight of sample, b = final weight after drying

Specific gravity

Specific gravity was determined using a pycnometer. The empty pycnometer was weighed, then filled with water and weighed again to calculate water density. After emptying, the pycnometer was filled with extract and weighed. Specific gravity of 0.5 g extract was calculated as:

$$\text{Specific gravity} = \frac{\text{extract density}}{\text{water density}}$$

Antihyperglycemic Effect Test

The study began with a 7-day acclimatization period for male mice under laboratory conditions with standardized care and diet. Mice were marked for identification. After 16 h of fasting with free access to water, body weight and blood glucose levels were measured to confirm normoglycemia (62–175 mg/dL). Only normoglycemic mice were included. Diabetes was induced by intraperitoneal injection of alloxan monohydrate dissolved in aqua for injection. After 72 h, blood glucose levels were reassessed via tail vein sampling; mice with levels >175 mg/dL were classified as diabetic. Forty diabetic male mice were randomly assigned to eight groups (n = 5) and treated orally for 8 days as follows:

Group I: Negative control (0.5% Na.CMC)

Group II: Positive control (glibenclamide 0.006 mg/g BW)

Group III: MLE (0.65 mg/kg BW)

Group IV: PLE (1.05 mg/kg BW)

Group V: MLE 0.65 mg/kg BW + PLE 1.05 mg/kg BW

Group VI: MLE 0.315 mg/kg BW + PLE 0.525 mg/kg BW

Group VII: MLE 0.315 mg/kg BW + PLE 1.05 mg/kg BW

Group VIII: MLE 0.65 mg/kg BW + PLE 0.525 mg/kg BW

Blood glucose was measured every 2 days for 8 days via tail vein sampling.

Data Processing and Analysis

Blood glucose levels of alloxan-induced diabetic mice treated with different extract combinations were measured. The most effective concentration in reducing blood glucose was determined. Data were analyzed using one-way ANOVA with SPSS version 26.0.

Table 1. Phytochemical screening results of MLE and PLE.

Phytochemical	Reagent	Observation	MLE	PLE
Alkaloids	Mayer's reagent	Yellow precipitate	+	–
	Wagner's reagent	Brown precipitate	+	+
	Dragendorff's reagent	Orange precipitate	+	+
Flavonoids	Mg + Concentrated HCl	Formation of red, yellow, or orange solution	+	–
Saponins	Hot water + HCl	Formation of foam up to 10 cm	+	+
Tannins	Ferric chloride (FeCl3)	Formation of bluish-black color	+	+
Steroids	Chloroform + Liebermann-Burchard test	No green color formed	+	–
Terpenoids	Chloroform + Liebermann-Burchard test	Formation of purple ring	–	+

RESULTS AND DISCUSSION

The samples used were Moringa leaf extract (MLE) and Paliasa leaf extract (PLE). MLE was prepared by maceration using 70% ethanol, selected for its higher polarity to enhance the extraction of polar compounds. Paliasa leaves were harvested in the morning following Dwinatari (2015) to minimize evaporation and transpiration. The leaves were washed, chopped, dried, and macerated with 96% ethanol for 3 × 24 h with daily solvent replacement. The filtrates were concentrated using a rotary evaporator and further dried. Extract yields were 67.54% for MLE and 67.48% for PLE from 500 g of raw material, meeting the Ministry of Health of Indonesia standards (≥3.6% for 500 g).

Both MLE and PLE underwent phytochemical screening to identify their secondary metabolite content. As shown in Table 1, MLE contains alkaloids, flavonoids, saponins, tannins, and steroids, consistent with the findings of Tutik et al. (2018), while PLE contains alkaloids, saponins, tannins, steroids, and terpenoids, in accordance with the results reported by Shella Desiana et al. (2018). Phytochemical screening is a preliminary method that uses specific reagents to detect classes of chemical constituents such as flavonoids, alkaloids, tannins, saponins, and terpenoids (Putri et al., 2013).

Flavonoids are widely recognized for their antioxidant properties, which protect cells from oxidative stress by neutralizing reactive oxygen species (ROS), enhancing the body's antioxidant systems, and thereby helping to manage hyperglycemic conditions (Rajanandh et al., 2012). These compounds also regulate blood glucose levels by modulating enzymes involved in carbohydrate metabolism (Brahmachari, 2011). As phenolic antioxidants, flavonoids protect pancreatic β-cells from oxidative damage, stimulate insulin secretion via the

Tables 2. Identity and quality parameters of MLE and PLE

Section	Parameter	MLE	PLE
Specific Parameters	Name of Extract	Moringa Leaf Extract	Paliasa Leaf Extract
	Latin Name	*Moringa oleifera* L.	*Kleinhovia hospita* L.
	Plant Part Used	Leaf	Leaf
	Color	Brown	Dark green
	Odor	Aromatic	Distinctive
	Taste	Bitter	Bitter
	Form	Powder	Thick Extract
	Water-Soluble Compounds (%)	33.3%	10%
	Ethanol-Soluble Compounds (%)	55.3%	46%
Non-Specific Parameters	Drying Loss (%)	1.9%	1.29%
	Specific Gravity of 5% Solution (g/mL)	1.02	1.21

cAMP signaling cascade, and inhibit ROS generation through metal ion chelation in Fenton and Haber-Weiss reactions (Shahidi & Wanasundara, 1992).

Beyond flavonoids, PLE also contains triterpenoids, which exhibit antidiabetic effects through activation of AMP-activated protein kinase (AMPK), promoting glucose uptake into cells (Yuliana et al., 2013). Phenolic compounds contribute further by preventing the formation of advanced glycation end products (AGEs) through inhibition of monosaccharide-induced protein glycation.

In vivo evidence supports these findings: administration of PLE at 750 mg/kg body weight significantly reduced blood glucose levels in alloxan-induced hyperglycemic Wistar rats. This effect is comparable to that of *Moringa oleifera* ethanol extract administered at 450 mg/kg body weight in diabetic rats induced by alloxan and fed a high-fat diet (Chinedu, Alani, & Olaide, 2015).

Following the identification of secondary metabolites, each extract was subjected to quality standardization tests to ensure compliance with raw material standards for herbal medicines, as outlined by the Indonesian Ministry of Health (Ministry of Health RI , 2000). The standardization covered specific parameters such as extract identity, organoleptic characteristics, and soluble compound levels, as well as non-specific parameters including loss on drying and specific gravity. Identity testing confirmed that the materials used were indeed derived from *Moringa* and *Paliasa* leaves, as shown in Table 2.

Organoleptic tests, using the five senses, assessed shape, color, odor, and taste of extracts to verify quality. Water-soluble and ethanol-soluble compound contents were determined to measure polar and semi-

polar constituents, respectively (Saefudin et al., 2011). Results indicated that the water-soluble compound content of Moringa leaf extract was 33.3%, while ethanol-soluble was 55.3%. PLE showed 10% water-soluble and 46% ethanol-soluble compound content. These values conform with the Indonesian Herbal Pharmacopoeia (Ministry of Health RI, 2017), which sets specific compound content limits for Moringa leaf extract (≤4.9% water-soluble and ≤5.3% ethanol-soluble) and PLE (≤18.2% water-soluble and ≥6.6% ethanol-soluble).

The higher water-soluble compound content in Moringa extract indicates greater polarity, while Paliasa extract is more semi-polar due to its higher ethanol-soluble content. However, soluble compound content does not directly influence active compound efficacy (Saefudin et al., 2011).

Loss on drying tests aim to show the amount of extract lost or evaporated during drying, critical for maintaining quality and preventing fungal growth (Safitri, 2015). Loss on drying for MLE was 1.9%, and for PLE 1.29%, both meeting BPOM standards of <10% (Ministry of Health RI, 2017).

Specific gravity tests, performed by diluting the sample to 5% with distilled water, provide insight into extract chemical content. Results showed 1.02 g/mL for Moringa and 1.21 g/mL for Paliasa extracts (Saifudin et al., 2017).

Next, antihyperglycemic testing was performed on 40 male mice (Mus musculus) divided into eight groups with five mice each. Treatments included Moringa extract at 0.63 mg/kg BW, Paliasa extract at 1.05 mg/kg BW, combinations of both extracts at different doses, Na.CMC 0.5% (negative control), and glibenclamide 5 mg (positive control). Treatment lasted 8 days, with blood glucose levels measured after induction and on days 4, 6, and 8 post-treatment. Glucose levels were initially measured before and after alloxan induction to confirm hyperglycemia (>176 mg/dL) (Nugrahani, 2015). Blood glucose was measured using AutoCheck® glucometer strips.

Mice were acclimatized to the environment to reduce stress before treatment (Katzung, 2010) and fasted for 16 hours prior to dosing to empty stomach contents that could affect results, but were allowed water (Lolok et al., 2019). Alloxan induction was administered intraperitoneally twice every two days to directly target the pancreas and induce beta-cell damage, consistent with its diabetogenic effect (Kumalasari et al., 2019). Blood glucose was measured 2 days after induction to confirm hyperglycemia.

Statistical analysis (Table 3) showed that fasting and post-induction blood glucose levels increased significantly. According to Nomura (1975) as cited in Fitrianingsih et

Table 3. Mean blood glucose levels (mg/dL)

Treatment Group	Before Induction	After Induction	Day 4	Day 6	Day 8
KN	100 ± 14.70	225 ± 26.91	223.8 ± 24.40	226 ± 23.53	227.6 ± 27.80
KP	122 ± 20.51	239.8 ± 31.70	132 ± 34.30	105.4 ± 22.26	92.8 ± 22.67
MLE	110.2 ± 17.85	244.8 ± 23.46	118.8 ± 17.78	100 ± 5.09	82.2 ± 15.22
PLE	99.2 ± 19.30	213.8 ± 6.41	134.4 ± 18.63	119 ± 10.55	97.8 ± 19.12
EC1	96 ± 8.68	254.2 ± 19.30	119.4 ± 14.29	84 ± 3.21	68.8 ± 4.43
EC2	101.8 ± 13.64	322.8 ± 54.96	149.8 ± 39.34	88.4 ± 11.37	72.6 ± 1.92
EC3	111.2 ± 16.55	243.6 ± 35.70	142.2 ± 31.72	115.4 ± 15.91	93.8 ± 12.07
EC4	107 ± 9.66	239.4 ± 39.76	120.8 ± 30.97	98 ± 20.16	79.6 ± 18.51

Notes: KN (normal control), KP (glibenclamide), MLE (*Moringa oleifera* extract), PLE (*Kleinhovia hospita* extract), and EC1–EC4 (combinations of MLE and PLE at varying doses or ratios).

al. (2012), normal blood glucose levels in mice range from 62.8 to 176 mg/dL; however, alloxan induction raised glucose levels well above this threshold, confirming hyperglycemia. The 0.5% Na.CMC negative control showed no hypoglycemic effect, as expected since it is pharmacologically inert. Glibenclamide, the positive control, effectively lowered blood glucose by stimulating insulin secretion through the closure of ATP-sensitive potassium channels on pancreatic β-cell membranes, causing membrane depolarization and calcium influx, which triggers insulin release (Suherman, 2007).

Male mice were selected to avoid the confounding effects of estrogen fluctuations during the estrous cycle and to reduce stress-related variability; additionally, male mice provide physiological similarities to humans, ease of maintenance, availability, and cost-effectiveness (Pribadi, 2008; Hamzah et al., 2014). Following induction, mice were assigned to treatment groups, and glucose levels were monitored over the treatment period. All treatment groups, except the negative control, showed reductions in blood glucose likely reflecting the metabolic clearance of glucose (Jangga, 2016). The positive control (glibenclamide) demonstrated glucose reductions comparable to those seen in treatment groups.

Importantly, the combination groups, particularly EC1 (MLE 0.63 mg/kg BW + PLE 1.05 mg/kg BW), exhibited the greatest glucose-lowering effect throughout days 4, 6, and 8 (Table 4). This suggests a potential synergistic interaction between bioactive compounds in *Moringa oleifera* and

Table 4. Mean percentage reduction in blood glucose levels in mice

Treatment Group	Day 4 (%)	Day 6 (%)	Day 8 (%)
KN	0.53 ± 10.71	-0.44 ± 10.32	-1.15 ± 12.25
KP	44.96 ± 14.30*	56.04 ± 9.49*	61.29 ± 9.73*
MLE	51.47 ± 9.91*	59.15 ± 2.08*	66.44 ± 6.23*
PLE	37.14 ± 8.72*	44.34 ± 4.94*	54.25 ± 8.95*
EC1	53.02 ± 5.62*	66.95 ± 1.91*	72.92 ± 1.74*
EC2	53.61 ± 12.20*	72.61 ± 3.52*	77.50 ± 0.59*
EC3	41.62 ± 9.90*	52.63 ± 6.53*	61.49 ± 4.96*
EC4	49.56 ± 12.94*	59.06 ± 8.42*	66.74 ± 7.73*

Notes: KN (normal control), KP (glibenclamide), MLE (Moringa oleifera extract), PLE (Kleinhovia hospita extract), and EC1–EC4 (combinations of MLE and PLE at varying doses or ratios). * Statistically significant compared to negative control (KN), $p < 0.05$ (one-way ANOVA followed by Tukey post hoc test).

Kleinhovia hospita extracts. Moringa oleifera is rich in flavonoids such as quercetin and chlorogenic acid, known for their insulin-sensitizing and antioxidant properties, while Kleinhovia hospita contains flavonoids and saponins that may enhance peripheral glucose uptake or inhibit hepatic gluconeogenesis (Ghosh et al., 2012).

The efficacy of these combinations was comparable to glibenclamide, indicating the potential for plant-based polyherbal formulations as alternative or adjunctive therapies for type 2 diabetes, especially in regions where access to pharmaceuticals is limited or side effects are a concern. However, further studies are needed to elucidate the exact mechanisms involved, including insulin secretion, insulin sensitivity, and pancreatic β-cell regeneration, through biomolecular and histological assays. Additionally, toxicological assessments and pharmacokinetic profiling will be essential to confirm safety and optimize dosing.

While this study utilized male mice to reduce variability, future research including female mice would be valuable to explore sex-specific responses to these extracts. Moreover, dose-response studies could identify the optimal combination ratios for maximum therapeutic benefit.

Normality and homogeneity tests confirmed the data were suitable for One-Way ANOVA using SPSS 26.0 ($p > 0.05$). The ANOVA results showed significant differences between groups on days 4, 6, and 8 ($p = 0.000$), and post hoc Tukey HSD tests indicated that all treatment groups (MLE, PLE, combination doses EC1–EC4, and positive control) differed significantly from the negative control (Na.CMC) on each day (Table 4). These findings confirm the antihyperglycemic efficacy of Moringa oleifera leaf extract, Kleinhovia hospita leaf extract, and their combinations in alloxan-induced hyperglycemic mice.

CONCLUSION

Based on this study, the combination of

MLE and PLE effectively lowers blood glucose in alloxan-induced diabetic male mice, with the optimal dose of 0.63 mg/kg BW MLE and 1.05 mg/kg BW PLE. The combined treatment was more effective than single extracts, suggesting synergistic effects. Further research should investigate different testing methods, mechanisms of action, safety, long-term effects, dose optimization, and sex-specific responses to fully confirm their therapeutic potential.

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