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EVALUATION OF THE HYPOGLYCEMIC EFFECTS OF AQUEOUS LEAF EXTRACTS OF *AZADIRACHTA INDICA* (NEEM) AND *EMBLICA OFFICINALIS* (AMLA) IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

Usaini A. Khalil*[1], Vaibhavi Patel[2], Mukhtar Y. Bebeji[3], Mubarak G. Bello[4], Maryam L. Ammani[5], Rabiu N. Danraka[6] and Aliyu G. Musa[7]

[1]Health Services Directorate, Northwest University Kano, Kano State, Nigeria.
[2]Department of Pharmacology, Arihant School of Pharmacy & Bio-research Institute, Adalaj, Gandhinagar, India.
[3]Department of Pharmacy Technician, Bichi Academy college of Health Science and Technology, Kano State, Nigeria.
[4]Department of Pharmaceutics and Industrial Pharmacy, Kaduna State University, Kaduna 800244, Nigeria.
[5]Department of Pharmacology and Toxicology, Kaduna State University, Kaduna, Nigeria.
[6]Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria.
[7]Department of Pharmacology and Therapeutics, Bayero University Kano, Nigeria.

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***Corresponding Author: Usaini A. Khalil**
Health Services Directorate, Northwest University Kano, Kano State, Nigeria.
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ABSTRACT

Introduction: Diabetes mellitus is a chronic and debilitating health condition associated with significant morbidity and mortality worldwide. Neem and Amla leaves are used traditionally to manage patients with diabetic symptoms. Thus, scientific evaluations of these herbal remedies is highly recommended. **Aim**: The present study was carried out to evaluate the hypoglycemic effects of aqueous leave extracts of Neem, Amla, and their combination in streptozotocin-induced diabetic rats. **Experimental methods**: Oral Glucose Tolerance Test (OGTT) was done by inducing a hyperglycemic state via the administration of glucose solution (2mg/kg). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (55 mg/kg). A single dose of neem (400mg/kg), amla (400mg/kg), and their combination (400mg/kg), as well as metformin 100mg/kg, were administered orally to hyperglycemic rats in both OGTT and STZ induced rats. **Results:** Administration of neem (400mg/kg), amla (400mg/kg), and their combination (400mg/kg), as well as metformin 100mg/kg orally have shown a significant reduction in blood sugar level ($p < 0.05$) in both OGTT and STZ-induced diabetic rats. Hypoglycemic effects were shown best in combined neem-amla extract than the individual doses of either plant. A significant difference ($p < 0.01$) was observed between the diabetic control group and the groups treated with the combined extracts and metformin. **Conclusion**: The outcome of this study revealed that the extracts of neem and amla possess hypoglycemic effects and thus, hold potential for use as antidiabetic agents. Therefore, the mechanisms through which these plants exert their hypoglyecemic effects should be elucidated.

KEYWORDS: Diabetes, Amla, Neem, OGTT, Streptozotocin.

1.0 INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by high blood glucose levels, associated with other manifestations.[1] It encompasses a group of chronic metabolic disorders characterized by persistent hyperglycemia, resulting from a complete or relative deficiency in insulin secretion or action.[2–4] The disorder affects the metabolism of carbohydrates, fats, and proteins, impacting a large portion of the global population.[5] The metabolic abnormalities lead to symptoms of polyurea (frequent urination), polydypsia (excessive thrust), and polyphagia (excessive hunger).[6] It can be classified into several types, primarily based on the underlying causes and characteristics of the disease. The main types include: Type 1 Diabetes (T1DM), Type 2 Diabetes (T2DM), and Gestational Diabetes (GDM).[7]

The global prevalence of diabetes rose from 4% in 1995 to 5.4% of the world's population in 2025.[3] Thus, around 470 million people worldwide are suffering from diabetes, with around 90% having T2DM. Projections indicate that the number of adults living with diabetes will reach 643 million by 2030.[8] This figure is expected to rise to about 700 million by 2045.[9] This escalating epidemic poses a serious threat to public health in both developed and developing countries.[5]

Uncontrolled diabetes is associated with a variety of long-term complications, including heart disease, stroke, kidney failure, retinopathy, nerve damage, neuropathy, atherosclerosis, chronic infections, immune deficiency, and peripheral vascular disease.[10] This lead to growing interest in discovering new anti-diabetic agents that are more affordable, more effective, and have fewer side effects. This interest stems from the high costs and predictable adverse reactions associated with many existing synthetic oral hypoglycemic agents. In both developing and some developed countries, the over-the-counter use of polyherbal products is quite common, with manufacturers often claiming that these remedies can provide a complete cure for diabetes mellitus.[2]

Since time immemorial, medicinal plants have played a crucial role in healthcare systems worldwide, particularly in developing countries. It is estimated that about 80% of the population in these regions including Africa relies on traditional medicine, primarily herbal remedies, for their essential healthcare and socio-economic needs.[10] Traditional medicine often utilizes combinations of multiple herbs or herbal extracts, potentially leading to synergistic effects.[2] Such combinations may influence multiple metabolic pathways, thereby enhancing therapeutic efficacy and reducing the risk of side effects.[11]

Neem: A Miraculous Tree of the Tropics, commonly known as "Indian lilac" or "Margosa," is an evergreen tree originating from the Indian subcontinent, now widely distributed across tropical and subtropical regions, including Sri Lanka, Pakistan, Bangladesh, Burma, and parts of Iran where it is called *"Cherish"* or *Azad derakht* in Persian. It is also the official tree of Sindh Province in Pakistan and is highly valued worldwide for its medicinal and ecological benefits.[12–14] The United Nations has recognized neem as the "Tree of the 21st Century," owing to its exceptional properties and cultural significance.[15] Its leaves are recognized for their broad spectrum of medicinal properties, particularly their antioxidant, anti-inflammatory, and blood sugar-regulating effects.[16,17] Key compounds in Neem, include flavonoids, tannins, and alkaloids, are believed to contribute to its anti-diabetic action.[2,18]

Amla, scientifically known as *Phyllanthus emblica or Emblica officinalis*, is a prominent plant in traditional Indian medicine, Ayurveda. It belongs to the Euphorbiaceae family and is widely distributed across tropical and subtropical regions, including India, Pakistan, Sri Lanka, Southeast Asia, China, and Malaysia.[12,19] Commonly referred to as

Indian gooseberry or *amalaki* in Sanskrit, amla is renowned for its extensive medicinal and nutritional properties.[18,20] Its leaves, known for their high content of Vitamin C and polyphenols, exhibit strong antioxidant activity.[11] These properties help regulate blood glucose, reduce oxidative stress, and improve lipid metabolism.[16] Amla's bioactive compounds like ellagic acid, gallic acid, and emblicanin are thought to play a significant role in its anti-diabetic effects.[2,12] Decoction prepared from the leaves and seeds is used in the treatment of diabetes.[16]

The concept of combining herbs is grounded in the idea that different plants offer complementary benefits, allowing for a more holistic approach to disease management. Thus, exploring the antihyperglycemic potential of Neem and Amla, both individually and in combination, could provide insights into more effective, multi-targeted treatment strategies, blending traditional wisdom with modern scientific validation. This approach aligns with the growing interest in phytomedicine as a complementary or alternative option to conventional antidiabetic therapies.[10]

In this study, we assess the antihyperglycemic activity of aqueous extracts of Neem and Amla leaves individually and evaluate the efficacy of their combination in reducing blood glucose levels. We also compare the results with Metformin, a standard drug for diabetes management.

2.0 MATERIALS AND METHOD

2.1 Experimental Animals

The study utilized Wistar rats of both sexes, weighing 150-200 grams. These rats were sourced from the Animal House, Department of Pharmacology, Arihant School of Pharmacy and Bioresearch Institute, Adalaj, Gandhinagar, India. They were provided with filtered tap water and food pellets ad libitum. The rats were kept in a well-ventilated room, housed in plastic cages with stainless steel wire mesh covers, and the cage floors were lined with wood bedding for comfort.

2.2 Drugs, Chemicals, and Equipment

Streptozotocin (Concept Technology, Ahmedabad, India) Metformin (Local pharmacy in India) Diagnostic Kits (Span Diagnostic Limited, India), Glucometer (Capricorn Scientific, India) Spirit and Alcohol (Local market). Others include; Distilled Water, 5% Glucose Solution, Normal Saline, Sterile Syringes and Needles, a Mixer, Filter paper, Collection Tubes, a Stopwatch, a Digital Weighing Balance, Animal Cages, Markers, an Incubator, Rotary Vacuum, Heating Mantle.

2.3 Collection of Plants and Identiification

The leaves were purchased from a local market in India. The leaves were identified and authenticated in the Department of Pharmacognosy, Arihant School of Pharmacy and Bioresearch Institute, Adalaj, Gujarat, India. A voucher numbers were assigned by comparing with an already deposited specimens; Amla (GJ/ASP&BRI/2024/0034) and Neem (GJ/ASP&BRI/2024/0035).

2.4 Preparation of Neem and Amla Aqeous Extract

The fresh leaves of each plant were washed separately with tap water and dried at room temperature for 2-3 days. After drying, they were grounded into a fine powder using a mixer.

A total of 100 grams of powdered leaves from each plant were soaked in 100 mL of distilled water for 24 hours. After filtration, the solvent was evaporated using a rotary evaporator to obtain a semi-solid extract. Each semi-solid extract was then freeze-dried to produce the final solid extract. These extracts were labeled as Amla leaf extract (ALE) and

Neem leaf extract (NLE).

2.5 Induction of Diabetes

Diabetes was induced in Wistar rats through a single intraperitoneal (i.p) injection of STZ. The STZ was dissolved in 0.1M citrate buffer (PH 4.5) and administered at a dose of 55 mg/kg body weight. To protect the STZ from light degradation, the solution was wrapped in aluminum foil. A total of 32 Wistar rats were fasted for 12 hours before the STZ injection. Diabetes was confirmed 72 hours post-injection by measuring blood glucose levels. Blood samples were collected from the lateral tail vein of each rat, and glucose levels were evaluated. Rats with blood glucose levels above 250 mg/dL were classified as diabetic and included in the study. To prevent hypoglycemic shock, the rats were provided with a 5% glucose solution to consume overnight after the STZ injection. Non-diabetic rats were excluded from the study.

2.6 Experimental Design

2.6.1 OGTT

Rats were given the following treatment in this study.

Group 1: Normal saline 1ml/kg

Group 2: Glucose (2mg/kg)

Group 3: Glucose (2mg/kg) + 400 mg/kg ALE

Group 4: Glucose (2mg/kg) + 400 mg/kg NLE

Group 5: Glucose (2mg/kg) + 400 mg/kg aqueous extract of both plants.

Group 6: Glucose (2mg/kg) + Metformin (100mg/kg)

Overnight fasted normal animals were divided into 6 groups, each group containing 5 rats. All Animals (except group 1) were administered glucose (2g/kg) orally by gastric intubation. Groups 3, 4, and 5 were orally treated with a single dose of 400 mg/kg body weight ALE, 400 mg/kg body weight NLE, and 400 mg/kg body weight aqueous extract of both plants respectively 30 minutes before administration of glucose solution. Group 6 received Metformin 100mg/kg and the control animals were administered normal saline. Blood samples were withdrawn from the tail vein by needle puncture at different time intervals of 30 minutes between four readings (0, 30, 60, 90, and 120 min).

2.6.2 STZ Induced Diabetes

Rats were given the following treatment in this study.

Group A: Control (Normal saline, 1ml/kg)

Group B: Diabetic (STZ) (55mg/kg)

Group C: Diabetic (STZ) (55mg/kg) + 400 mg/kg ALE.

Group D: Diabetic (STZ) (55mg/kg) + 400 mg/kg NLE.

Group E: Diabetic (STZ) (55mg/kg) + 400 mg/kg of both extracts.

Group F: Diabetic (STZ) (55mg/kg) + Metformin (100mg/kg).

The animals were divided into six groups, each consisting of five rats. Group A, serving as the non-diabetic control, received only distilled water and regular food without any STZ injection. The remaining rats were injected with STZ to induce diabetes and then divided into five groups (Groups B, C, D, E, and F). Group B (negative control) received STZ alone. Group C was treated with STZ along with 400 mg/kg body weight ALE. Group D received STZ and 400 mg/kg

body weight of NLE. Group E was administered a combination of 400 mg/kg body weight of both ALE and NLE following STZ induction. Group F (positive control) was treated with a standard dose of 100 mg/kg of Metformin. These treatments were given orally once daily for 15 days. Blood samples were collected from overnight-fasted animals on Days 1, 7, and 15 to measure blood glucose levels.

2.7 Ethical Approval

The project was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation of Animals). The study was conducted according to the rules and regulations provided by the University Research and Ethics Document.

2.8 Statistical Analysis

Results were expressed as Mean ± Standard Error of Means (Mean ± S.E.M). The data were analyzed using one-way ANOVA (Single-factor) followed by Bonferroni post hoc test for multiple comparisons. P-values less than 0.05 were considered statistically significant. The analysis was done using excel sheet version 2016.

3.0 RESULTS

3.1 Hypoglycemic Effect of Aqueous Leaf Extracts of Neem and Amla in OGTT

Compared to the negative control group, ALE (400 mg/kg), NLE (400 mg/kg), ALE+NLE (400 mg/kg), and metformin (100 mg/kg) significantly ($p < 0.05$) lowered the blood glucose levels. Besides, there were no significant ($p > 0.01$) difference between the metformin group and the extract pre-treated groups. Outlines the changes in fasting blood glucose levels during the OGTT. A significant reduction in fasting blood glucose levels was observed between the groups ($p < 0.05$). At 120-minute, blood glucose levels were: ALE**:** 129.5 ± 1.10 mg/dL, NLE: 132.7 ± 1.10 mg/d, Combination of ALE and NLE: 109.9 ± 0.62 mg/dL, Metformin: 102.22 ± 0.94 mg/d. The values are lower when compared with the diabetic control group. No statistically significance difference ($p > 0.01$) was observed between the metformin-treated group and the group where the combined extract was administered. The effects of neem and amla extracts IN OGTT were displayed in table 1.

3.2 Effects of Neem and Amla Extracts on STZ-induced Diabetic Rats

In STZ-induced diabetic rats, there was a significant ($p < 0.05$) decrease in fasting blood glucose levels between the groups. Groups C, D, E, and F exhibited a continuous decrease, while Groups A and B showed an increase in glucose levels on the 7th day and 15th day respectively. On the 15th day, the hypoglycemic activity was: Neem extract (Group D): 172.60 ± 1.69 mg/dL, Amla extract (Group C): 180.20 ± 0.69 mg/dL, Combination of extracts (Group E): 130.40 ± 1.16 mg/dL, Metformin (Group F): 124.60 ± 1.43 mg/dL. The combination of ALE and NLE exhibited higher hypoglycemic activity than individual extract, while metformin showed the greatest effect. A significant difference ($p < 0.01$) was observed between the diabetic control group and the groups treated with the combined extracts and metformin. Additionally, no statistically significant difference ($p > 0.05$) was observed between the metformin-treated group and the ALE + NLE group (Table 2).

Table 1: Hypoglycemic Effect of Aqueous Leaf Extracts of Neem and Amla in OGTT.

Groups	Drugs	Dose (mg/Kg)	Blood glucose (mg/dl)				
			0 hr	30 min	60 min	90 min	120 min
1	Saline	1	82.33±2.58	81.99±2.78	80.33±1.52	78.30±1.80	79.33±1.19
2	Glucose	2	80.22±1.80	140±0.86	139.55±0.44	137.60±0.57	133.7 ±1.4
3	Glucose+ALE	2+400	82.12±2.01	141.1±0.58	138.3±0.80	136.8±1.08	129.5±1.10
4	Glucose+ NLE	2+400	81.42±3.56	140.6±0.68	139.0±0.70	137.33±1.2	132.7±1.10
5	Glucose+ALE+NLE	2+200+200	81.99±2.95	135.2±2.58	132.23±1.7	116.0±0.5	102.22±0.94
6	Glucose+Metformin	2+100	80.55±1.77	136.44±0.77	129.3±0.97	115.0±0.70	102.22±0.94

Values are expressed as Mean ± S.E.M. Data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test, n=5.

P-Value= 0.021838

α= 0.01

Bonferroni (Post-Hoc test)		
Groups	**P-value(T-test)**	**Significance**
Group 1 vs Group 2	0.004222504	YES
Group 2 vs Group 5	0.510208345	NO
Group 2 vs Group 6	0.401722006	NO
Group 5 vs Group 6	0.824987816	NO
Group 5 vs Group 1	0.006237788	YES

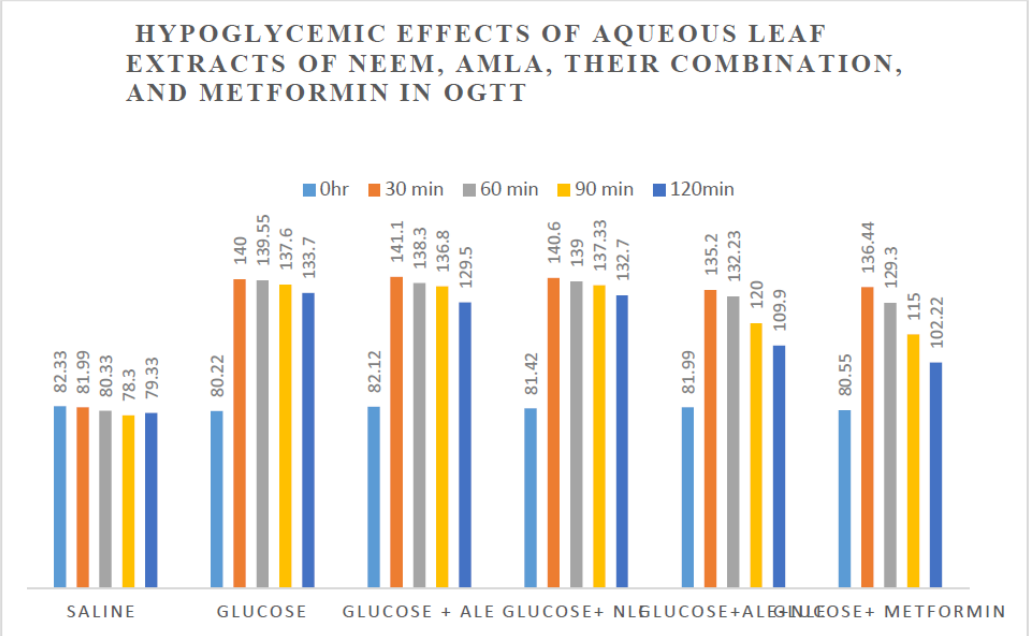

Fig. 1: Hypoglycemic Effects of Aqueous Leaf Extracts of Neem, Amla, their combination, and Metformin in OGTT (mg/dl).

Table 2: Hypoglycemic Effects of Aqueous Leaf Extracts of Neem, Amla, their combination and Metformin in *STZ-induced* diabetic rats.

Groups	Drugs	Dose (mg/Kg)	Blood glucose (mg/dl)		
			1st day	7th day	15th day
A	Saline	1	82.00±0.70	84.80±0.86	81.00±0.70
B	STZ	55	290.70±1.20	287.60±1.16	297.90±2.13
C	STZ+ALE	55 +400	249.0±2.06	231.20±3.05	180.20±0.69
D	STZ+ NLE	55+400	240.20±1.06	229.60±1.77	130.40±1.16
E	STZ+ALE+NLE	55+200+200	215.90±1.2	157.60±1.02	130.40±1.16
F	STZ+ Metformin	55+100	210.80±0.86	150.80±1.15	124.60±1.43

P-value = 0.000131

Bonferroni (post-hoc test)		
Groups	**P-value(T-test)**	**Significance**
Group A vs Group B	3.497E-07	TRUE
Group B vs Group E	0.008130905	TRUE
Group B vs Group F	0.007184052	TRUE
Group E vs Group F	0.848367041	FALSE
Group E vs Group A	0.027748817	FALSE

α= 0.01

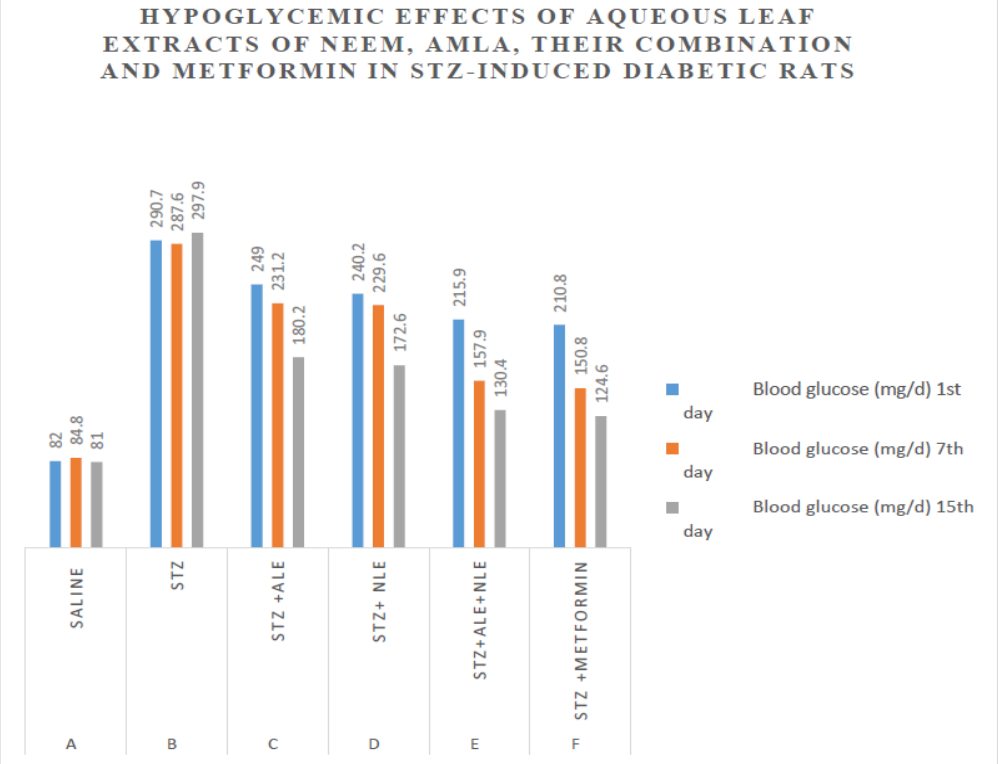

Fig. 2: Hypoglycemic Effects of Aqueous Leaf Extracts of Neem, Amla, their combination and Metformin in STZ-induced diabetic rats.

4.0 DISCUSSION

Medicinal plants like neem and amla, rich in bioactive secondary metabolites, are valuable sources of therapeutic agents. Their integration into traditional and modern medicine emphasizes their role as versatile and potent natural remedies. OGTT and STZ-induced diabetic animals are commonly employed to screen new agents with hypoglycemic potentials. Rafiq *et al*. demonstrated that a single dose of STZ can induce a pronounced diabetic symptoms.[21] However, subsequent treatment with metformin significantly lower blood glucose levels.[22] Neem is widely recognized for its diverse therapeutic properties, including antioxidant, anti- inflammatory, antihyperglycemic, anticarcinogenic, and immunomodulatory effects.[13,23] These attributes have made neem a vital component in traditional medicine and a focus of modern scientific research.

In the current study, the ability of neem extract to remarkably lower the blood glucose levels is suggestive of its potential as hypoglycemic agent. These findings are consistent with earlier studies conducted. Another study attributed the hypoglycemic properties of neem to the inhibition of α-amylase, a key enzyme in carbohydrate metabolism.[24] Moreover, the hypoglycemic effect of neem extract was thought to be due to the presence of flavonoids it contains.[33-35] Amla is also used in Ayurveda to treat a wide range of ailments and enhance overall health. It is widely acknowledged

that every part of the amla plant holds medicinal value. Research highlights its diverse therapeutic properties, including analgesic, antitussive, cardioprotective, cytoprotective, immunomodulatory, chemopreventive, antioxidant, memory-enhancing, anticancer, and antidiabetic effects. Traditionally, Ayurveda utilized its roots, leaves, stems, and fruits, with the fruit being particularly noted for its remarkable health benefits. The leaves and bark are rich in tannins, adding to their medicinal properties. Amla is especially renowned as a key herb for managing diabetes, treating bleeding disorders, enhancing stamina, and promoting strength. Its status as one of the richest natural sources of vitamin C underscores its role as a powerful antioxidant.[25–28]

Research has further confirmed amla's pharmacological benefits, such as its antioxidant, gastroprotective, chemopreventive, hypolipidemic, antiviral, antibacterial, antiulcerogenic, hepatoprotective, cardiotonic, antipyretic, and antidiabetic activities.[2] Clinical studies demonstrated that amla supplementation can effectively reduce fasting and postprandial blood glucose levels and improve HbA1c levels, making it a beneficial agent in diabetes management.[18,20]

Experimental studies reveal that oral administration of ALE at 100 mg/kg body weight significantly lowers blood glucose levels in both normal and alloxan-induced diabetic rats (120 mg/kg body weight) within four hours. Additionally, amla has been shown to delay the onset of diabetic cataracts and inhibit aldose reductase (AR), an enzyme associated with diabetes- related complications.[27,29,30] Traditional uses include decoctions of amla leaves and seeds for managing diabetes mellitus.[31] Our findings align with these studies, demonstrating that reduce blood glucose levels in STZ-induced diabetic rats. This further validates amla's potent antihyperglycemic effects and supports its traditional use as a natural remedy for diabetes.

Moreover, this study demonstrated that the combination of Amla and Neem leaf extracts resulted in increased hypoglycemic effect compared to individual treatments in both OGTT and STZ-induced diabetic rats. As described by Vega-López *et al.*, hyperglycemia is the most critical condition in diabetes, often accompanied by a reduction in body weight.[32] Choudhury *et al.* emphasized that maintaining blood glucose levels and body weight are key parameters for evaluating the efficacy of an antidiabetic agent. This study showed that the combined extracts significantly reduced blood glucose levels while maintaining body weight, meeting these essential criteria.[33]

The findings also suggested that the combination treatment effectively reduced glucose intolerance and exhibited robust hypoglycemic properties. These results support the pharmacological relevance of these herbal plants and validate their use in traditional and ethnotherapeutic practices for regulating and managing diabetes. It further shows that combination treatment offers superior benefits for diabetes management compared to individual extracts. These findings highlight the potential of this treatment strategy for improving glycemic control and supporting holistic diabetes care.

5.0 CONCLUSION

The study demonstrated that aqueous leaf extracts of Neem and Amla, individually and in combination, possess significant hypoglycemic and antihyperglycemic activities in normoglycemic and STZ-induced diabetic rats. The combination of the extract was effective in reducing blood glucose levels and maintaining body weight, with effects comparable to metformin, highlighting its potential as a prophylactic and therapeutic agent for diabetic management. Further research is needed to elucidate their mechanisms of action, explore long- term effects, and optimize formulations for clinical use.

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