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EVALUATION OF THE ANTI-HYPERGLYCEMIC POTENTIAL OF AQUEOUS NEEM (*AZADIRACHTA INDICA*) LEAF EXTRACT IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is a class of metabolic disorder characterized by a persistent hyperglycemic state caused by defect in insulin synthesis, insulin action, or combination of both. Medicinal plants have played important role in healthcare systems worldwide, particularly in developing countries. Neem (*Azadirachta indica)* is celebrated for its diverse applications, including its medicinal, agricultural, and therapeutic uses. Its leaves have been recognized for a broad spectrum of medicinal properties, particularly antioxidant, anti-inflammatory, and blood sugar-regulating effects. This study evaluated the antihyperglycemic activity of its aqueous leave extracts. 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). Aqueous neem leaf extract (NLE) produced statistically significant changes in blood sugar levels ($P<0.05$) in both OGTT and STZ-induced diabetic rats. The study demonstrated that aqueous NLE possesses hypoglycemic and antihyperglycemic activities in normoglycemic and STZ-induced diabetic rats, highlighting its potential as a prophylactic and therapeutic agent for diabetes management.

KEYWORDS: Azadirachta Indica (Neem), Diabetes Mellitus, Streptozotocin, OGTT, Blood Glucose.

INTRODUCTION

Diabetes mellitus encompasses a group of chronic metabolic illnesses marked by persistent hyperglycemia, resulting from a total or relative deficiency in insulin secretion or action.[1–3] According to the American Diabetes Association

(ADA) 2024, diabetes is a chronic health disorder that affects the body's ability to process blood glucose (sugar), leading to high blood glucose levels over time. It is a class of metabolic illnesses marked by a persistent hyperglycemic state caused by insulin production, insulin action, or both.[4-5]

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), Gestational Diabetes (GDM), and Other Specific Types (These may include monogenic diabetes, diabetes due to diseases of the exocrine pancreas, and drug-induced diabetes).[4] Symptoms include Polyurea, Polydipsia, and polyphagia.[5]

Globally, the prevalence of diabetes is expected to rise from 4% in 1995 to 5.4% by 2025.[2] Currently, 463 million people worldwide are suffering from diabetes, with around 90% having T2DM. It has been predicted, there will be 643 million adults with diabetes by 2030.[6] This figure is expected to rise to approximately 700 million by 2045.[7] This escalating epidemic poses a severe threat to public health in both developed and developing countries.[8]

Complications in numerous organs can be caused by uncontrolled diabetes. Damage to small and large blood vessels, as well as nerves results in impaired vision and kidney dysfunction, heart attacks, strokes, and amputation of the lower limb.[1,11]

There has been a 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.[1]

Medicinal plants have played an important 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.[9]

Neem is celebrated for its diverse applications, including its medicinal, agricultural, and therapeutic uses. In India, it is revered as the "divine tree," "life-giving tree," and "village pharmacy." Across different cultures, it is also called by names such as *Nim* (Hindi, Bengali, Assamese), *Azaddarakhulhind* (Arabic), *Baypay* (Malaysia), and *Dogonyaro* (Nigeria).[10,11] The United Nations has recognized neem as the "Tree of the 21st Century," owing to its exceptional properties and cultural significance.[12]

Neem leaves are recognized for their broad spectrum of medicinal properties, particularly their antioxidant, anti-inflammatory, and blood sugar-regulating effects.[13,14] Key compounds in Neem, such as flavonoids, tannins, and alkaloids, are believed to contribute to its anti-diabetic action.[1,15]

Taxonomic Classification[16–18]

Common Name: Neem

Botanical Name: *Azadirachta indica*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida
Order: Sapindales
Family: Meliaceae
Genus: Azadirachta
Species: A. indica

In this study, we assess the antihyperglycemic activity of aqueous leaf extracts of Neem in Normal and Streptozotocin-Induced Diabetic rats with the view of finding out its possible integration as a complementary medicine in the management of diabetes mellitus.

MATERIALS AND METHODS

Experimental animals

The study utilized Wistar rats of both sexes, averaging between 150-200 grams in weight. These rats were sourced from the Animal House at the Department of Pharmacology, Arihant School of Pharmacy and Bioresearch Institute, Adalaj, Gandhinagar, India. They were provided with filtered tap water and given 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.

Plant materials

The leaves of *Azadirachta indica* (Neem) were obtained from the local market in Sector 24, Gandhinagar, Gujarat, India, for the study.

Drugs, Chemicals, Kits, and Equipment

Streptozotocin (STZ): Sourced from Concept Technology, Ahmedabad, Diagnostic Kits: Obtained from Span Diagnostic Limited, Metformin Tablets (500 mg): Purchased from a local pharmacy. Glucometer: Acquired from Capricorn Scientific, Spirit and Alcohol: Procured from the 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, a Rotary Vacuum, a Heating Mantle.

Preparation of Neem aqueous extract

The fresh leaves of the neem plant were washed thoroughly with tap water and dried at room temperature for 2-3 days. After drying, they were ground into a fine powder using a mixer. 100 grams of powdered leaves 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 powdered form.

Induction of Diabetes

Diabetes was induced in Wistar rats through a single intraperitoneal (IP) 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, based on the individual weight of each rat. To protect the STZ from light degradation, the solution was wrapped in aluminium foil.

A total of 24 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 using a one-touch glucometer based on the glucose oxidase method. 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.

In the treatment phase, the diabetic rats were orally administered the plant extract for 15 days. The control group, consisting of 5 rats, received normal saline as a vehicle.

Experimental design

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 NLE

Group 4: Glucose (2mg/kg) + Metformin (100mg/kg)

Overnight fasted normal animals were divided into 4 groups each group containing 5 rats. All Animals (except group 1) were administered glucose (2mg/kg) orally by gastric intubation. Groups 3 and 4 were orally treated with a single dose of 400 mg/kg body weight NLE and Metformin 100mg/kg respectively 30 minutes before administration of glucose solution.

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). The level of glucose was estimated by using glucose-oxidase peroxidase reactive strips and a glucometer.

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 NLE for 15 days.

Group D: Diabetic (STZ) (55mg/kg) + Metformin (100mg/kg) for 15 days.

The animals were divided into four groups, each consisting of five rats. Group A, serving as the non-diabetic control, received only normal saline and regular food without any STZ injection. The remaining rats were injected with STZ to induce diabetes and then divided into three groups (Groups B, C, and D). Group B, the negative control, received STZ but no additional treatment. Group C was treated with STZ along with 400 mg/kg body weight NLE. Group D, the 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 using a glucometer.

Statistical analysis

Data are expressed as Mean ± Standard Error of Means (Mean ± SEM). One-way ANOVA (Single factor) with Post hoc (Bonferroni) test was used for statistical analysis. P values below 0.05 and 0.01 were considered statistically significant for ANOVA and Post hoc tests respectively.

RESULTS AND DISCUSSION

Table 1: Hypoglycaemic Effect of Aqueous Leaf Extracts of *Azadirachta indica* (Neem) and Metformin in OGTT (Mean ± S.E.M) (n=5).

Groups	Drugs	Dose (mg/kg)	Blood glucose (mg/dl)				
			0hr	30 min	60 min	90 min	120min
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+ NLE	2+400	81.42±3.56	140.6±0.68	139.0±0.70	137.33±1.2	132.7±1.10
4	Glucose+ Metformin	2+100	80.55±1.77	136.44±0.77	129.3±0.97	115.0±0.70	102.22±0.94

P value = 0.01092

Table 2: Hypoglycaemic Effects of Aqueous Leaf Extracts of *Azadirachta indica* (Neem) and Metformin in *STZ-induced* diabetic rats (Mean ± S.E.M) (n=5)

Group	Drugs (mg/kg)	Dose	Blood glucose (mg/d)		
			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+ NLE	55+400	240.20±1.06	229.60±1.77	172.60±1.69
D	STZ +Metformin	55+100	210.80±0.86	150.80±1.15	124.60±1.43

P value = 0.002952

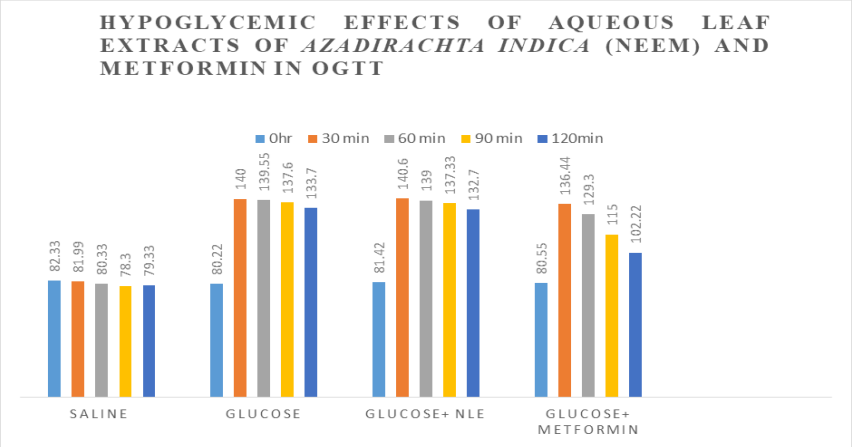

Fig. 1: Hypoglycemic Effects of Aqueous Leaf Extracts of *Azadirachta indica* (Neem) and Metformin in OGTT (mg/dl).

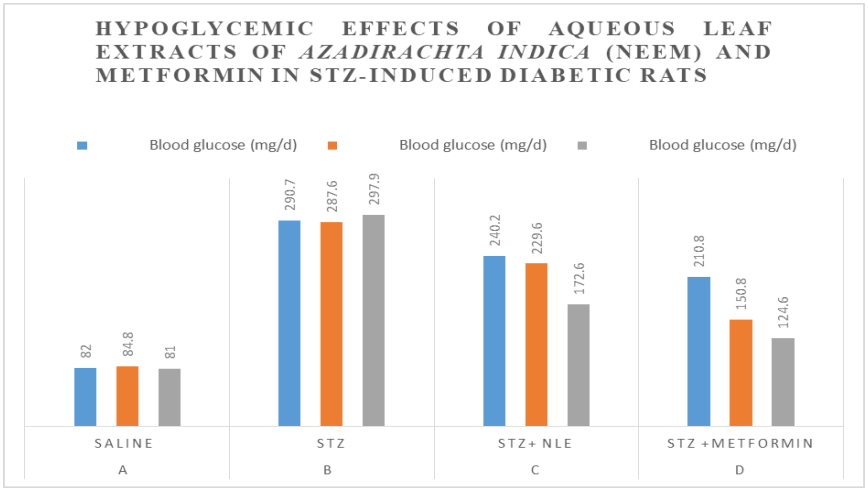

Fig. 2: Hypoglycemic Effects of Aqueous Leaf Extracts of *Azadirachta indica* (Neem) and Metformin in STZ-induced diabetic rat.

In table 1 above, a significant reduction in fasting blood glucose levels was observed between the groups ($P < 0.05$). At 120 minutes, blood glucose levels were: **Saline group:** 79.33±1.19 mg/dl, **Diabetic control:** 133.7 ±1.4 mg/dl, **NLE:** 132.7 ± 1.10 mg/dl, **Metformin:** 102.22 ± 0.94 mg/d. The values are lower when compared with the diabetic control group. No statistically significant difference ($P>0.01$) was observed between the metformin-treated group and the group where the NLE was administered. The rats from group 3 and group 4 showed a greater ability to metabolise glucose as depicted by the reduction in their blood glucose levels at different intervals throughout the entire period of 120 min.

In STZ-induced diabetic rats (table 2), there is a significant decrease in fasting blood glucose levels ($P< 0.05$) between the groups. Groups C (STZ + NLE) and D (STZ + Metformin) 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 Saline group: 81.00±0.70, STZ group: 297.90±2.13, NLE group: 172.60 ± 1.69 mg/dL, Metformin group: 124.60 ± 1.43 mg/dL. While metformin shows the greatest effect with a significant difference when compared with diabetic control, no significant difference ($P > 0.01$) was observed between the NLE-treated group and the diabetic control group. Additionally, no significant difference ($P>0.01$) was observed between the metformin-treated group and the NLE-treated group. We observed that the rats from Group C treated with NLE showed distinctly reduced blood glucose levels compared with the diabetic control group. Rats in Groups C and D treated with NLE and Metformin had the lowest blood glucose levels throughout the entire 15-day period.

This data demonstrates that a significant reduction in fasting blood glucose levels was observed between the groups ($P < 0.05$) in both OGTT and STZ-induced diabetes. This shows that aqueous neem leaf extract at a dose of 400 mg/kg and metformin at 100mg/kg lower fasting blood glucose levels indicating their hypoglycemic activities.

Neem is widely recognized for its diverse therapeutic properties, including antioxidant, anti-inflammatory, antidiabetic, antihyperglycemic, anticarcinogenic, and immunomodulatory effects. These attributes have made neem a vital component in traditional medicine and a focus of modern scientific research.[11,16]

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. A study attributed the hypoglycemic properties of neem to the inhibition of α-amylase, a key enzyme in carbohydrate metabolism.[19] Moreover, the hypoglycemic effect of neem extract was thought to be due to the presence of flavonoids it contains.[20]

CONCLUSION

The study demonstrated that aqueous leaf extracts of Neem possess significant hypoglycemic and antihyperglycemic activities in normoglycemic and STZ-induced diabetic rats, highlighting its potential as a prophylactic and therapeutic agent for diabetes management. This study demonstrates the potential of neem extracts as effective alternatives for managing diabetes mellitus and underscores their significance in developing sustainable and innovative treatments for hyperglycemia.

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