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Antihyperglycemic Activity of *Plumeria alba Linn*. Leaves Extracts in Streptozotocin-nicotinamide Induced Diabetic Rats

Jyoti Nanda[1,2]*, Munesh Mani[1], Shanti Bhushan Mishra[3] and Neeraj Verma[4]

[1]Pharmacy Academy, IFTM University, Moradabad, U.P., India.
[2]School Of Pharmaceutical Sciences, CSJM University, Kanpur, U.P., India.
[3]United Institute of Pharmacy, Prayagraj, U.P., India.
[4]Hygia Institute of Pharmaceutical Education and Research, Lucknow, U.P., India.
*Corresponding Author E-mail:jyotinanda954@gmail.com

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Diabetes mellitus is a metabolic condition wherein the body's blood sugar levels are elevated. Plant-based treatments are less toxic than allopathic pharmaceuticals. Plumeria alba Linn (Apocynaceae) is a popular medicinal plant that contains flavonoids, tannins, alkaloids, terpenoids, and antioxidants. The antihyperglycemic action of Plumeria alba Linn. leaves, on the other hand, has yet to be determined. A Streptozotocin-nicotinamide-induced Type II diabetes model was used to elaborate on the hypoglycemic activity of ethanolic and aqueous leaf extractives of Plumeria alba, which were compared to the diabetic control group. Anti-diabetic activity was tested on albino Wistar rats. Normal and experimental rats were given ethanolic and aqueous leaf extracts at 250 mg/kg b.w. and 500 mg/kg b.w. for 21 days, and the effect on blood sugar levels was measured. In comparison to the diabetic control group, the ethanolic extract at a dose of 500 mg/kg b.w. exhibits a highly significant ($p<0.001$) reduction in blood glucose levels.

Keywords: Antidiabetic activity; Blood glucose level; plant extract; Plumeria alba; Streptozotocin- nicotinamide.

Hyperglycemia, altered lipid, carbohydrate, and protein metabolism, and an elevated risk of vascular disease consequences are all characteristics of diabetes mellitus. Type II diabetes affects the vast majority of diabetic people. According to the WHO Global Diabetes Report 2016, the number of people living with hyperglycemia has nearly doubled to 422 million[3]. There are diabetic patients who have insulin insufficiency or resistance, as well as excessive glucagonemia. Diabetic kidney disease (DKD) patients are more likely to develop end-stage kidney disease (ESKD), cardiovascular disease, increased mortality, lower quality of life, and higher healthcare costs[5].

Furthermore, type II diabetes is increasingly being diagnosed at an alarmingly high rate in preadolescents and teenagers. The main cause of complications of diabetes mellitus is high-end blood glucose levels in the form of sorbitol in the body. Reactive oxygen species induce a complexing agent called advanced glycation end-product (AGE), which may convert a normal glucose level to hyperglycemia level [4].Modern pharmacotherapeutics, such as insulin, biguanides, sulfonylureas, and thiazolidinediones, have emerged as a result of the advancement of modern medicine [8].The important biologically active substances are hydroxylated polyphenolic

compounds, specifically flavonoids, which provide powerful antioxidant activity and are important in the treatment of diabetes and the prevention of diabetic angiopathies[10]. Imeglimin is a first-in-class novel oral antidiabetic agent for type 2 diabetes that was recently approved by Japan's Pharmaceuticals and Medical Devices Agency as a new oral antidiabetic drug in June 2021[2].

Oral hypoglycemic medications, which are commonly used to treat diabetes, have been shown to have a number of side effects [7]. In a study with hairless mice, phototoxicity to sulphonamide-derived oral anti-diabetes medications such as glibenclamide, glipizide, glymidine, tolazamide, and tolbutamide was observed. As a result, research continues to focus on active traditional herbals that have well-established scientific evidence for blood sugar-lowering activity; diabetes therapy with herbs has become a popular option around the world[6].

With an impressive range of medicinal and biological properties, *Plumeria alba* Linn, also known as White Champa, has been used in the ancient medicine systems of several civilizations for cardiotonic, purgative, diuretic, rheumatic, and hypotensive activity. The latex of the plant is used to treat herpes, scabies, and ulcers on the skin [1]. The bark is bruised and used to cover hard tumours like a plaster. Leaves are lanceolate to oblanceolate, with fragrant white blooms in corymbose fascicles. Based on a review of the literature, the antidiabetic effect of this plant in rats has only been documented in a few studies; hence, this study has been recommended. The goal of the study was to see how ethanolic and aqueous extracts of *Plumeria alba* leaves affected BSL in streptozotocin-nicotinamide-induced rats [9,11].

MATERIALS AND METHODS

Plant material and extract preparation

Fresh leaves of *Plumeria alba* Linn. were obtained locally in Lucknow, Uttar Pradesh, and confirmed and identified by Dr. Lal Babu Chaudhary, Senior Principal Scientist and curator of the Herbarium. Herbarium's plant accession number 105283 is deposited at the CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India, for future reference.

Fresh leaves were dried in the shade before being ground with a mechanical grinder. The powdered dried drug of *P. alba* leaves (1 kg) used in a Soxhlet apparatus was successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol, and distilled water. At the Hygia Institute of Pharmaceutical Education and Research in Lucknow, India, collected extracts were concentrated in a vacuum rotary evaporator and dried. The yields for the ethanolic extract of *P. alba* (EEPA) leaves and the aqueous extract of *P. alba* (AEPA) leaves were computed in relation to the whole amount of drug employed in extraction.

Drugs and chemicals

Drugs, Streptozotocin (Calbiochem, Mumbai, India), Nicotinamide (HiMedia, Mumbai, India), Glibenclamide pure powder (API as a gift sample from Sigma-Aldrich Ltd., Mumbai, India), and an Accu-chek blood glucose meter and strips (Roche, India) were obtained.

Preparation of extract solutions

Each extract was made to achieve a concentration of 100 $mgml^{-1}$ in D.W., with doses of 250 $mgkg^{-1}$b.w. and 500 $mgkg^{-1}$ b.w. respectively.

Experimental animals

Animal House, United Institute of Pharmacy, Prayagraj, U.P., India, provided healthy rats of both sexes. For a 7-day acclimation phase, animals were kept in experimental conditions at ambient temperature with a day-night cycle. The experimental study followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory animal experimentation, and the United Institute of Pharmacy's Institutional Animal Ethics Committee (Registration Number 1451/PO/Re/S/11/CPCSEA) had approved the research protocol (UIP/IAEC/Nov.-2020/06). Rats weighing 150–167 g of either sex were used as inclusion criteria.

Acute toxicity study of extracts

Acute toxicity studies are commonly conducted in experimental animals to determine the LD_{50} value, which is used to determine the therapeutic dose, according to the OECD-423 guidelines. Singular dose of EEPA and AEPA extracts (5$mgkg^{-1}$, 50 $mgkg^{-1}$, 300 $mgkg^{-1}$, and 2000 $mgkg^{-1}$, 5000 $mgkg^{-1}$) in suitable H_2O administered orally by gavage to various groups of rats (3 each).

For subsequent pharmacological activity, doses of 250 $mgkg^{-1}$b.w. and 500 $mgkg^{-1}$b.w. decided on LD_{50} estimation.

Streptozotocin Nicotinamide induced hyperglycemia in rats

In animal studies, Streptozotocin-nicotinamide caused hyperglycemia. The trial lasted 21 days and involved repeated oral administration [8].Group I is non-diabetic group. In overnight fasting rats from Groups II to VII, hyperglycemia is caused by injecting a singular dose of nicotinamide (120 mg /kg b.w) intraperitoneally, followed by a singular dose of Streptozotocin (60 mg/ kg b.w) once [6,12]. A glucose diet is given to rats to prevent fatal hypoglycemia. Animals were tested for hypoglycemic activity 72 hours after receiving STZ-NA injections by monitoring their blood sugar level (BSL) with a glucometer.

Experimental design for anti-diabetic activity

Streptozotocin Nicotinamide-induced hyperglycemia (STZNAD) in rats of either sex was placed into six groups (6 rats in each group) and 1 non-diabetic group (ND). Throughout the trial, animals had unrestricted access to water and feed. AEPA 250 mg/kg b.w., AEPA 500 mg/kg b.w., EEPA 250 mg/kg b.w., and EEPA 500 mg/kg b.w. were administered once a day for 21 days.

Group I-NC: Non Diabetic rats received Distill Water and served as normal control.

Group II-DC: Streptozotocin Nicotinamide caused hyperglycemia rats received Distill Water and served as diabetic control.

Group III-STD: Streptozotocin Nicotinamide caused hyperglycemia rats received Glibenclamide 600 µg/kg.

Group IV- AEPA 250mg/kg: Streptozotocin

Table 1. Effect of several *Plumeria alba* extracts on blood sugar levels in Streptozotocin Nicotinamide diabetic animals

Groups (n)	0 Day	7th Day	14th Day	21st Day
NC	81.5±9.48	81.16±9.42	80.0±8.56	81.83±8.74
DC	280.16±30.15^{z}	273±33.29^{z}	268.0±32.05^{z}	259.33±21.70^{z}
STD	271.33±31.76	264.83±31.91	121.33±20.34^{c}	94.66±7.95^{c}
AEPA 250mg/kg	278.5±31.16	272.83±33.39	126.33±14.62^{b}	112.0±4.32^{c}
AEPA 500mg/kg	276.5±31.58	270.5±32.83	123.66±14.24^{b}	106.83±4.02^{c}
EEPA 250 mg/kg	273.5±29.60	267.5±31.27	121.16±14.32^{c}	101.66±8.33^{c}
EEPA 500mg/kg	272.5±28.31	265.83±31.09	117.5±14.78^{c}	98.33±9.28^{c}

(Source-self developed using MS Office Excel 2010)
Each group's data is represented by the mean and standard deviation of six rats. $^{b}p < 0.01$ and $^{c}p < 0.001$ against DC group. $^{z}p < 0.001$ against NC.

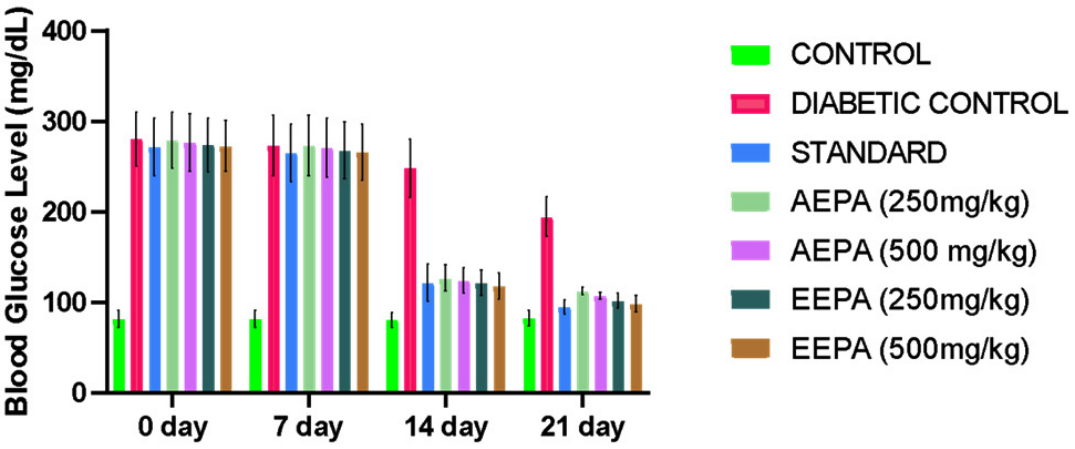

Fig.1. Blood glucose level of *Plumeria alba* extracts
(Source- self developed using Prism 9.3.1)

Nicotinamide caused hyperglycemia rats received AEPA 250mg/kg

Group V- AEPA 500mg/kg: Streptozotocin Nicotinamide caused hyperglycemia rats received AEPA 500mg/kg

Group VI- EEPA 250mg/kg: Streptozotocin Nicotinamide caused hyperglycemia rats received EEPA 250mg/kg

Group VII- EEPA 500mg/kg: Streptozotocin Nicotinamide caused hyperglycemia rats received EEPA 500mg/kg

Monitoring of blood sugar level during treatment

Blood samples were taken from rats by cutting the tips of their tails, and blood sugar levels were measured by a blood glucose strip with an Accu Check glucometer. Every week during the study period, BSL readings were taken on the 0th, 7th, 14th, and 21st days.

Statistical analysis

The mean and standard deviation were used to represent the data. Graph Pad Prism 9.3.1 software was used to analyze the data. The one-way Analysis of Variance (ANOVA) and the Newman–Keuls test were used to evaluate all data. Statistically, a result of ($p < 0.001$) was found more significant.

RESULTS

Yield value of extracts in percentage

In terms of the total amount of powdered drug used for extraction, the percentage yields were 21.81% (EEPA) and 19.11% (AEPA) respectively.

Acute toxicity study of *Plumeria alba* leaves extracts

At the highest dose (5000 mg/kg b.w.), the acute toxicity investigation found immorality within 4 hours and 14 days after oral administration of AEPA and EEPA.

Effect of *Plumeria alba* leaves extracts on diabetes

When compared to the diabetic group, oral treatment with AEPA (250 mg/kg b.w. and 500 mg/kg b.w.) and EEPA (250 mg/kg b.w. and 500 mg/kg b.w.) resulted in a remarkable diminution in blood sugar level (mg/dL). In comparison to the DC, a dose of 500 mg/kg b.w. of ethanolic extract of *Plumeria alba* exhibited a more remarkable result ($p<0.001$) (Table 1).

DISCUSSION

The combination of the Streptozotocin-nicotinamide model precludes pancreatic beta cells from becoming insulin-deficient; making it suited for type II diabetes. When STZ is injected alone, it completely deteriorates pancreatic beta cells and causes insulin deficiency; however, injecting NA first protects insulin-secreting pancreatic cells from the injurious effects of STZ [9].

An oral daily dose of *Plumeria alba* aqueous extract and ethanolic extract (250 mg/kg b.w. and 500 mg/kg b.w.) shows the impact on blood sugar levels in 21st days (Table 1). At the end of treatment, the blood sugar level reduced from 278.5 $mgdL^{-1}$ to 112.0 $mgdL^{-1}$ (59.78%) in rats treated at a dose of 250 mg/kg b.w. ($p < 0.001$) and blood glucose level reduced from 276.5 $mgdL^{-1}$ to 106.83 $mgdL^{-1}$, (61.36%) in rats treated at a dose of 500 mg/kg ($p<0.001$) orally aqueous extract.

Orally 250 $mgkg^{-1}$ of the ethanolic extract, the blood sugar level declined from 273.5 $mgdL^{-1}$ to 101.66 $mgdL^{-1}$ (62.82%) at 21 days ($p < 0.001$) and blood glucose level reduced from 272.5 $mgdL^{-1}$ to 98.33 $mgdL^{-1}$ (63.91%) in animals treated at a dose of 500 $mgkg^{-1}$ ($p<0.001$) ethanolic extract of *Plumeria alba*.

For Glibenclamide 600 μgkg^{-1} body weight, the sugar level reduced from 271.33 $mgdL^{-1}$ to 94.66 $mgdL^{-1}$ (65.11%) ($p < 0.001$). The effect of ethanolic extract 500 $mgkg^{-1}$ b.w. is more markable ($p<0.001$) than other extracts of *Plumeria alba.* (Fig no. 1)

The effect of Glibenclamide on glucose tolerance has been attributed to the increased activity of pancreatic beta-cells, resulting in the release of more insulin. As a result, the mechanism underlying the antihyperglycemic activity of *Plumeria alba* extracts shows an impact like insulin, most likely via peripheral glucose consumption or improved beta-cell glucose tolerance, culminating in enhanced insulin release [7].

CONCLUSION

We discovered that *Plumeria alba* extracts with anti-diabetic activity significantly reduced blood glucose levels. *Plumeria alba* has been shown to be a safe and effective anti-diabetic

medication. The effects of plant extracts on lipid profiles and antioxidant levels in hyperglycemic rats will be explored further.

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Conflicts of Interest

The authors declare no conflicts of interest.

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