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Research Article

Effect of Active Fraction Isolated from the Leaf Extract of *Allmania nodiflora* on Plasma Glucose Concentration and Lipid Profile in Streptozotocin-Induced Diabetic Rats

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Abstract

The current study's objective was to determine how active fractions from *Allmania nodiflora* leaves affected the lipid and serum glucose profiles in normal and diabetic rats. Streptozotocin (45 mg/kg) activated diabetes in wistar rats. Ethanolic extract of *Allmania nodiflora* (EEAN) leaf was given orally at doses of 200 and 400 mg/kg.p.o. Metformin (50 mg/kg p.o.) was administered as the usual anti-diabetic medication. When compared to normal control rats, EEAN considerably ($p < 0.05$) decreased the fasting blood glucose (FBG) level. In addition, column chromatography was used to isolate an active compound, which was given the innocuous name AN-1. A study of the hypoglycemic and hypolipidemic effects of AN-1 (100 mg/kg) was conducted. In diabetic rats, AN-1 significantly ($p < 0.05$) decreased FBG levels while simultaneously lowering cholesterol and triglycerides and raising HDL levels. This finding suggests that AN-1 has demonstrated anti-diabetic and anti-hyperlipidemic activity, and it offers a rational scientific justification for the use of anti-diabetic medications.

Keywords: Blood Glucose; *Allmania nodiflora*; Lipid Profile; GC-MS

Introduction

Diabetes is a complicated and multifaceted collection of diseases that affects how carbohydrate, fats, and proteins are metabolised and is defined by high fasting and postprandial blood sugar levels [1]. Diabetes mellitus is a condition where the body's capacity to create or react to the hormone insulin is compromised. As a result, there is improper carbohydrate metabolism and an increase in blood glucose levels. Type I (insulin dependent diabetes mellitus, IDDM) and type II (non-insulin dependent diabetic mellitus, NIDDM) are the two kinds of diabetes mellitus [2]. The immune system of the body damages the pancreas, which prevents the body from producing insulin, which is the cause of type 1 diabetes. The body is unable to produce insulin in type 1 diabetes. In type 2 diabetes, the body either becomes resistant to insulin or produces insufficient amounts of the hormone to lower blood sugar levels [3]. 90% of people with diabetes have type II diabetes, which is the most common kind of the disease. By the year 2025, it was predicted that 300 million people would be affected, up from the 1995 estimate of 135 million people [4]. The management of diabetes without any side effects continues to be a problem for the medical profession. Currently, a number of medications, including biguanides, sulfonylureas, and thiazolidenediones, are available to treat diabetes mellitus [5]. These medications pharmacokinetic characteristics, secondary failure rates, and associated side effects limit their therapeutic applications [6]. In order to control diabetic issues and help prevent long term complications, it is

therefore imperative to find a new class of compounds, which eventually prompts a constant quest for substitute medications [7]. The latest oral hypoglycemic compounds for the creation of pharmaceutical entities or as dietary supplements to existing therapies may be derived from medicinal plants [8]. Furthermore, research on hypoglycemic compounds from medicinal plants has grown increasingly crucial in light of the WHO's recommendation on diabetes mellitus. According to ethnobotanical information sources, 800 plants may have anti-diabetic properties [9]. Numerous experts working in the field of diabetes research have recently examined the therapeutic potential of numerous plant extracts [10]. Modern healthcare has new opportunities thanks to the logical creation of newer medications derived from traditional medicine [11]. Traditional Indian medicine practitioners in India frequently treat dysmenorrhea, muscle spasms, cough, discomfort, and poisoning with *Allmania nodiflora*, an herb from the amaranthaceae family. Due to the arrangement and structure of the leaves, the Chinese also refer to this plant as having "wings of a fly." Previous phytochemical studies on *Allmania nodiflora* were successful in isolating astragalin, cosmossiin, tectorigenin [12], 2-O-glucosylvitexi, 2-O-xylosylvitexi [13], vitexin, genistin, aliphatic alcohols, aliphatic acids, ursolic acid, oleanolic acid, campesterol, stigmasterol [14]. The goal of the current study was to assess the ability of several *Allmania nodiflora* extracts/fractions to lower blood sugar levels in order to identify the active ingredient responsible for the plant's anti-diabetic effects. The effects of the active ingredient (AN-1) from *Allmania nodiflora* leaf extract on normal and diabetic rats were examined in further research.

Methods

Drugs and chemicals

We bought streptozotocin (STZ) from Sigma Aldrich in Bangalore, India. Metformin, a common anti-diabetic medication, was purchased from Actavis Pharmaceutical in Chennai, India. For the extraction and phytochemical analysis of the components, analytical grade chemicals from S.D. Fine Chemicals, India, including different organic solvents (petroleum ether, ethyl acetate, and ethanol), were utilised.

Preparation of different plant extracts

The leaves of *Allmania nodiflora* were gathered in the Kalakatu forest in the Tirunelveli District of India. From a botanical survey of medicinal plants conducted by the Siddha Unit of the Government of India and authenticated by botanist Chelladurai in Palayamkottai, taxonomic identification was made. A specimen of a voucher is CCRAS-1154/2017. Fresh plant leaves were airtight-packed, temperature-controlled shade-dried into a fine powder. Then, using a continuous hot extraction method using the soxhlet apparatus at a temperature of 60°C, 500 g of the sample was extracted for 72 hours with each of the accelerating polarity solvents petroleum ether, ethyl acetate, and ethanol. Using a rotating evaporator and lowered pressure, the extracts were concentrated to a constant weight. The extracts were gathered and stored in a desiccator until they were needed for additional research.

Acute toxicity study

In accordance with OECD - 423 recommendations, an acute toxicity study was carried out. This study used a random selection procedure to choose six albino mice (n = 6) of either sex. The animals only had free access to water during their 4-hour fasting. Mortality was tracked for three days after oral administration of the different extract of *Allmania nodiflora* suspended in normal saline: tween 80 (95:5) at a dose of 5 mg/kg at first. When 5/6 or 6/6 animals died, the dose given was deemed hazardous. Out of six animals, less than four mice died, but the deadly impact was confirmed when the same amount was administered twice. The technique was then repeated with increasing doses such 50, 300, 1000, and 2000 mg/kg because the mortality was not seen the first time.

Animals

Each male wistar rat weighed 180–220 g, and they were procured from Aditya Bangalore Institute of Pharmacy Education and Research (ABIPER) in Bangalore, Karnataka, India. Prior approval was given by the Institutional Animal Ethics Committee (NO.1611/PO/a/12/CPCSEA) in accordance with the guidelines provided by the Committee for the Purpose of control and Supervision of Experiments on Animals (CPCSEA) of the Government of India. Rats were maintained on a 12-hour light/dark cycle in a temperature-controlled environment (20-25°C) during the experimental procedures, and rodent laboratory chow became access and water at will.

Induction of diabetes

50 mg/kg of STZ had been intravenously given into the fasting rats [15]. Prior to usage, the STZ was newly dissolved in citrate

buffer (0.01 M, pH 4.5) and kept on ice. Rats that exhibited Fasting blood glucose (FBG) concentrations of more than 150 mg/dl were considered diabetic and employed in the experiment one week following STZ injection.

The oral glucose tolerance test (OGTT) in normal and STZ induced diabetic rats

Rats with and without diabetes were placed into five groups of six each after an overnight fast. Only 1.0 ml of distilled water was given to the rats in Group I. *Allmania nodiflora* extracts were given orally to rats in groups II through IV at doses of 200 mg/kg each. Metformin 50 mg/kg was administered to rats in group V. Each rat received an oral dose of glucose (3.0 g/kg) 30 minutes later. In order to estimate the blood glucose level, blood samples (0.5-0.6 ml) were taken from the tail vein in frozen heparinized tubes at -30, 0, 30, 90, 120, and 210 min. Plasma was extracted after centrifugation (2000 g) and kept at 20°C. Diagnostic Kit's were used to measure the plasma glucose levels utilising the glucose oxidase-peroxidase method [16]. The ethanolic extract demonstrated the greatest reduction in FBG level among all the extracts, according to the results of the pharmacological screening. Hence EEAN subjected to isolate the active principle, column chromatography was used.

Isolation of active principle from ethanolic extract of *Allmania nodiflora* leaf

Allmania nodiflora (10g) ethanol extract was chromatographed on a silica gel column (60-120 mesh). In order of increasing polarity, the column was progressively eluted with petroleum ether, petroleum ether-chloroform mixture, and chloroform-methanol mixture in various proportions. Fractions with identical Retention factor values on the TLC were mixed, and at reduced pressure, they were evaporated to dryness. After eluting with chloroform: methanol (70:30), the primary active fraction (2.5g) was recovered. It was then purified by chromatography over a column of silica gel (100-200), yielding a yellow amorphous solid (2.0g) (75:25). By using GC-MS analysis, a single fraction was identified and given the obnoxious name AN-1.

Study of AN-1 on FBG and lipid profile in diabetic rats

Rats with and without diabetes were divided into four groups of six each. 1.0 ml of distilled water was given to the normal rats in group I. Rats with diabetes in Group II were given 1.0 ml of distilled water. Rats with diabetes in Group III received 100 mg/kg of AN-1. Rats with diabetes in Group IV got 50 mg/kg of metformin. For 21 days, oral treatment was given to all groups. The animals underwent a moderate ether anaesthesia to obtain blood samples from the retro orbital plexus at the conclusion of the experimental period after fasting for 8 hours the previous night. Using Span Diagnostic kits (5X100 ml), plasma was isolated, and the FBG level was assessed using the glucose oxidase-peroxides method. Using span diagnostic kits in chemical under techniques, cholesterol level was evaluated by the enzymatic method [17], triglyceride level by the enzymatic colorimetric method [18], and HDL level by the phosphotungstate method [19].

Identification of isolated fraction

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained in figure 1. The compounds discovered by GC-MS analysis in the current study thus demonstrated a variety of biological actions to create drugs of medicinal interest.

Figure 1: GC-Mass Spectrum of isolated fraction AN-1.

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance was used to conduct the statistical analysis (ANOVA). For mean comparisons, the least significant difference test was performed, and ($p < 0.05$) was regarded as statistically significant.

Results

Acute toxicity study

2000mg/kg of the extract was ingested as per OECD regulations orally to 6 mice. Effects were noticed for change in behavior for 3 days. Mice were observed for the behavioral effects 45mins after the administration of the extracts. There was no change in behavior or no abnormality in behavior was seen. The rats exhibited no mortality. It can be asserted that various extract of *Allmania nodiflora* was non-toxic up to 2000 mg/kg doses. Then 1/10th of the administered amount of drug was fixed for further studies as per OECD-423 guidelines.

Effect of different leaf extracts of *Allmania nodiflora* on FBG of normal and diabetic rats

In addition to the common medication Metformin (50 mg/kg), various extracts of *Allmania nodiflora* (200 mg/kg) were studied in normal and diabetic rats. EEAN significantly ($p < 0.05$) decreased plasma glucose concentration in normal and diabetic rats, similar to Metformin, as shown in tables 1 and 2.

Treatments	Time (min) before and after glucose administration					
	-30	0	30	90	150	210
Normal animals	72.3 ± 2.3	79.6 ± 2.1	183.2 ± 2.6	146.4 ± 2.8	95.8 ± 7.8	84.2 ± 6.4
Petroleum ether (200 mg/kg)	79.5 ± 1.2	72.5 ± 6.3	172.3 ± 4.4	111.3 ± 1.4	104.5 ± 3.2	92.3 ± 2.2
Ethyl acetate (200 mg/kg)	75.2 ± 1.7	69.4 ± 2.5	133.3 ± 2.8	112.4 ± 4.2	101.4 ± 2.3	91.5 ± 2.1
Ethanol (200 mg/kg)	72.4 ± 2.8	71.4 ± 1.6	120.2 ± 4.9	97.1 ± 1.5	93.6 ± 1.3*	82.5 ± 1.8*
Metformin (50 mg/kg)	75.5 ± 3.1	79.8 ± 2.3	121.1 ± 2.2	94.4 ± 1.2	83.9 ± 1.1*	75.3 ± 3.4*

Table 1: Effect of different *Allmania nodiflora* leaf extracts on plasma glucose concentration in normal rats.

Data are expressed as mean ± SEM. n= 6 rats per group. *p < 0.05, compared to normal control group.

Treatments	Time (min) before and after glucose administration					
	-30	0	30	90	150	210
Normal animals	154.8 ± 2.6	187.8 ± 2.1	224.1 ± 4.4	215.3 ± 3.9	168.3 ± 3.8	176.2 ± 1.4
Petroleumether (200 mg/kg)	134.5 ± 2.2	139.7 ± 2.9	225.6 ± 2.4	211.6 ± 2.4	163.7 ± 4.9	157.1 ± 2.8
Ethyl acetate (200 mg/kg)	155.9 ± 1.9	143.1 ± 2.7	219.3 ± 3.8	184.4 ± 3.4	163.4 ± 1.4	154.5 ± 2.8
Ethanol (200 mg/kg)	164.4 ± 1.6	153.4 ± 2.7	214.7 ± 3.9	175.1 ± 3.5	132.6 ± 3.3*	93.9 ± 1.6*
Metformin (50 mg/kg)	161.9 ± 2.1	162.8 ± 1.3	216.1 ± 2.5	133.4 ± 1.2*	125.9 ± 2.1*	75.8 ± 2.2*

Table 2: Effect of different *Allmania nodiflora* leaf extracts on plasma glucose concentration in diabetic rats.

Data are expressed as mean ± SEM. n = 6 rats per group. *p < 0.05, compared to diabetic control group.

Identification of isolated fraction and dose fixing

It was discovered that the active ingredient in EEAN was Anthracenol (Retention time-9.01) and mefenacet (Retention time-9.28) were confirmed to be the two components of AN-1 by GC-MS, as illustrated in figure 1. When given orally to rats, AN-1 did not cause any toxic symptoms, according to an acute toxicity study. The LD_{50} value, or deadly dose, was 1000 mg/kg body weight.

Effect of AN-1 on FBG and the Lipid Profile of Diabetic Rats

In diabetic rats, AN-1 significantly ($p < 0.05$) reduced FBG, similar to Metformin, as seen in table 3. Additionally, AN-1 resulted in a significant ($p < 0.05$) drop in triglyceride levels and a significant ($p < 0.05$) increase in HDL. With regard to cholesterol levels, the effect was minimal.

Treatment	Fasting plasma glucose (mg/dl)			Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)
	0 day	10 day	21 day			
Normal control	62.8 ± 5.9	89.9 ± 2.2	85.3 ± 3.5	91.5 ± 1.3	69.6 ± 2.4	44.2 ± 2.4
Diabetes alone	259.7 ± 2.8	217.6 ± 3.5	189.7 ± 2.4	82.5 ± 2.4	115.6 ± 4.8	37.3 ± 2.1
Diabetes + AN-1 (100 mg/kg)	242.6 ± 2.1	176.3 ± 2.5*	158.2 ± 3.1*	85.6 ± 2.6	62.6 ± 5.5*	44.7 ± 3.3*
Diabetes + Metformin(50 mg/kg)	233.6 ± 1.8	169.8 ± 4.5*	149.8 ± 2.8*	82.3 ± 2.8	92.6 ± 2.6	45.3 ± 1.1*

Table 3: Effect of oral administration of isolated fraction (AN-1) on plasma glucose concentration, cholesterol, triglyceride, and HDL for 21 days.

Data are expressed as mean ± SEM. n = 6 rats per group. *$p < 0.05$, compared to diabetic control group.

Discussion and Conclusion

Streptozotocin treatment in rats resulted in the fast death of pancreatic cells, impairing insulin release in response to glucose stimulation and leading to insulin resistance, both of which are indicators of diabetes. Plant extracts are effective in treating moderate streptozotocin-induced diabetes because they have hypoglycemic effects that are often dependent on the extent of pancreatic-cell damage [20]. As seen in diabetic rats, increased blood glucose levels are typically associated by increased plasma cholesterol, triglyceride, and LDL levels as well as lower HDL levels [21]. It's possible that the unchecked activity of lipolytic hormones on fat deposits is what causes the prominent hyperlipidemia (increased lipid levels in the body) that defines the diabetic state [22]. The ethanol extract produced a significant drop in blood sugar comparable to metformin therapy among all the extracts examined. Gas chromatography was used to identify the components of isolated fraction AN-1 as anthracenol and mefenacet, with the compound peak being seen at 9.01 and 9.28 retention times in both compounds. Lowering blood sugar and changing the lipid profile (increasing HDL, lowering TGL, and cholesterol) are two effects of anthracenol and mefenacet. It may be advantageous to prevent diabetes complications given the improvements in lipid profiles seen in diabetic rats after receiving anthracenol and mefenacet therapy. Anthracenol and mefenacet from the ethanolic extract of the *Allmania nodiflora* leaf have been demonstrated in the research to significantly lower blood sugar and modify the lipid profile in diabetic rats. Further studies need to be finding of Anthracenol and mefenacet action for hypoglycaemic and hypolipidemic effect.

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Nil.

Conflict of Interest

Nil.

Authors' Contribution

The research was conceptualised by AD. VN carried out the analysis, implementation, and design. The data gathering was done by AD, VN, and BAV, who also helped with the manuscript's authoring.

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