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Original Research Article

Evaluation of *Amaranthus spinosus* leaf aqueous extract anti-diabetic activity in streptozotocin-induced diabetic rats

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Abstract

All across the world, especially in nations where access to the traditional treatment of diabetes mellitus is insufficient, plants and their bioactive elements are used to treat diabetes mellitus. The hypoglycemic properties of *Amaranthusspinosus* have been the subject of numerous reports. The goal of the current investigation was to determine whether *Amaranthusspinosus* leaf extract alone or in conjunction with the oral hypoglycemic medication glibenclamide had any anti-diabetic effects on STZ-induced diabetic rat models. For the purpose of inducing diabetes in albino rats weighing 180–200g, STZ (50 mg/kg, I.P.) was utilised.

The fasted diabetic rats were divided in to 5 groups of 6 animals each. Group I Normal control received distilled water 5 ml/kg body weight orally daily, Group II were treated with STZ 50mg/kg body weight induced diabetic rats were served as diabetic control, Group III i.e STZ induced diabetic rats were served as standard group and were treated with Glibenclamide at a dose of 0.5 mg/kg body weight orally daily. Group IV &Group V *Amaranthusspinosus* leaves extract was administered with 200mg/kg and 400mg/kg dose orally. This study was conducted over a period of 15 days with oral administration of drugs and the plant extract which was started on the 6th day of STZ treatment. The fasting blood glucose levels were determined on day 0, 5th, 10th, and 15th day by using glucometer. The ANOVA statistical test was used to analyse the data, and then the Dunnett's multiple comparison test. In STZ-induced diabetic rats fed with the aqueous extract of *Amaranthusspinosus,* all the test groups displayed significantly (P 0.001) reduced fasting blood glucose levels. However, the blood glucose level was significantly (P 0.001) decreased. The presence of flavonoids, tannins, steroids, and trepenoids in *Amaranthusspinosus*is thought to be responsible for its strong hypoglycemic activity.

Keywords: *Amaranthusspinosus (AS)*,Streptozotocin(STZ), Glibenclamide, Hypoglycemia & Diabetes mellitus.

Introduction

More than 2000 years ago, diabetes was first described. It has been a part of the development of modern medicine over the last 200 years. A lack of insulin secretion or a combination of insulin resistance and insufficient insulin secretion to make up for it are the two main causes of the syndrome, which is characterised by abnormal metabolism and inappropriate hyperglycemia[1].

Diabetes is a medical "iceberg" condition. 90% of cases globally are of type 2 diabetes, which is the most common type. Both industrialised and developing nations are seeing an epidemic of type 2 DM. The World Health Organisation (WHO) estimates that there are 180 million diabetics globally, with 300 million expected by 2025[2].According to WHO estimates, there were 19.4 million diabetics in India in 1995, and that number is expected to rise to 57.2 million by 2025.The updated estimate is 80.9 million by 2030. Since the ICMR research in 1970, which indicated a prevalence rate of 2.3% in the urban population and 1.5% in the rural population, the prevalence rates have been continuously rising[3].The prevalence of type-2 diabetes currently ranges from 10% to 18% among adult Indians living in urban areas, and there is evidence that this rate is rising in rural areas as well. Increases in life expectancy, obesity rates, and sedentary lifestyles have all contributed to a rise in diabetes prevalence. Genetics is one of the causes of this sharp increase. Genetic predisposition, urbanisation, ethnicity, insulin resistance, and central obesity are the causes of this sharp increase[4].Indians make up one in five diabetics worldwide, and one in five and one in ten in major cities like Mumbai[5].In order to treat associated conditions like dyslipidemia, hypertension, obesity, coronary heart disease, and

to screen for or manage complications of diabetes like retinopathy, cardiovascular disease, nephropathy, neuropathy, and other complications, the goals of therapy for diabetes mellitus include glycemic control through diet, lifestyle modification, regular exercise, medication, i.e., oral anti diabetic drugs, and insulin therapy[6].All across the world, especially in nations where access to the traditional treatment of diabetes mellitus is insufficient, plants and their bioactive elements are used to treat diabetes mellitus[7].There have been few studies on *Amaranthusspinosus*' hypoglycemic properties[8,9].

*Amaranthusspinosus*Linn. (Family Amaranthaceae), a very common Indian plant is known for its medicinal properties and commonly known as 'spiny amaranth' or 'pig weed', ''Kate waliChaulai (Kanatabhajii)" in 'Hindi", cultivated throughout in India, Sri Lanka and distributed throughout the tropics and warm temperate regions of Asia from Japan to Indonesia, the Pacific islands and Australia as a weed in cultivated as well as fallow lands. In Indian traditional system of medicine (Ayurveda) the plant is used as febrifuge, antipyretic, laxative and diuretic. Besides its culinary value, it is used to repute for treat digestible, bronchitis, appetizer, biliousness, galactagogue, haematinic, stomachic, nausea, flatulence, anorexia, blood diseases, burning sensation, leucorrhoea, leprosy and piles. Phytochemical investigations prove its importance as valuable medicinal plant. It is known as rich source of alkaloids, flavonoids, glycosides, phenolic acids, steroids, tannins, amino acids, terpenoids, lipids, saponin, betalain, b-sitosterol, stigmasterol, linoleic acid, rutin, catechuic tannins and carotenoids [10,11,12,13,14]. Amaranthusspinosus contains 7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside, a new coumaroyl flavone glycoside called spinoside, xylofuranosyl uracil, beta-D-ribofuranosyl adenine, beta-sitosterol glucoside, betaxanthin, betacyanin; gomphrenin, betanin and beta-carotene[15,16,17]. The leaves and stems also revealed contain hentriacontane, octacosanoid, α-spinasterol, saponin and fatty acids[11]. The roots contain α-spinasterolsoctacosanoate and saponin, viz. saponin of oleanolic acid[18].The studies on *A. spinosus*have been carried out by various researchers and a wide spectrum of its pharmacological actions have been explored which may include antidiabetic, antitumor, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, spermatogenic, antifertility, antimalarial, antioxidant properties, etc.

The primary drawback of modern medications (biguanides, sulfonylureas) is that they must be taken for the rest of one's life and result in side effects [19].The goal of the current investigation was to determine whether *AS* leaves extract alone or in combination with the oral hypoglycemic medication glibenclamide has an anti-diabetic effect on STZ-induced diabetic rat models.

Methods

Plant Material and Extract Preparation

Amaranthusspinosus leaves available locally, were identified and used for the study.After identification, *Amaranthusspinosus* leaves were obtained. The leaves were cleaned, dried in the shade, and grounded to coarse particles and aqueous extraction was carried out using with Soxhlet apparatus. The extract was filtered to remove the peeled particles. The residue was re-extracted with the same solvent. The extracts were pooled and concentrated under vacuum at 45°C[20].

In this investigation, adult albino rats (180-200 grams) of either sex were utilised. A total of 30 rats were divided into 5 groups of six each. Using polythene tubing sleeved on an 18–20 gauge blunted hypodermic needle, all of the medications were given to the animals orally. The animals were stabilised for a whole week at typical temperatures of 25°C, 60% relative humidity, and 12 hours of darkness and light. Since they had received. They had been given free accesses to standard pellet diet and water ad libitum. Experiments were conducted according to the ethical norms approved by the Animal Ethics Committee guidelines Shadan college of Pharmacy, Telangana.

Drugs and chemicals

Streptozotocin [21] - obtained from Sigma-Aldrich limited, Mumbai. To induce a stable hyperglycaemic state in rats, the dose of Streptozotocin suggested in literature is a single intraperitoneal injection at a dose of 50 and 60 mg/kg body weight. Hence, initially we administered 60 mg/kg intraperitoneal injection of streptozotocin to the animals and half of the animals died. Then, we tried 50 mg/kg injection and noted that all the animals developed diabetes with no mortality.

Glibenclamide - Tablets 80 mg - 5% Gum acacia-Vehicle for administrating the fine suspension of glibenclamide. Standard dose for glibenclamide is 0.5mg/kg. Glibenclamide (glyburide) belongs to sulfonylureas class of oral Hypoglycaemics [22].

Diabetes Mellitus Induction in Rats

Streptozotocin was stored at 4 - 8° C. It was dissolved insterile normal saline. It was always prepared freshly for immediate use. All rats were fasted overnight before diabetes was induced. STZ

was given in the dose of 50 mg/kg body weight, single intraperitoneal injection.

The animals were observed to be diabetic from the 3rd day onwards. The animals showing a blood glucose level of 200 mg/dl and above were considered diabetic and were included in the study.

Anti-Diabetic Experimental Design [23]

The fasting diabetic rats was separated into 6 groups of 6 animals each for the anti-diabetic trial.

Group I: Received distilled water 5ml/kg, orally.

Group II: STZ 50mg/kg body weight i.p induced diabetic rats were served as diabetic control.

Group III: STZ induced diabetic rats were served as standard group and were treated with Glibenclamide at a dose of 0.5 mg/kg, orally, daily.

Group IV: Diabetic rats – received *AS* leaves extract 200mg/kg, orally, daily.

Group V: Diabetic rats – received *AS* leaves extract 400mg/kg, orally, daily.

All the drugs were given orally as a single dose in the morning. The fasting blood glucose levels were determined on day 0, 5, 10, and 15.

Blood Collection Procedure [24]:

Rat tail vein blood was drawn to estimate blood glucose levels. After an overnight fast, fasting blood glucose levels were taken in all rats. After xylene was used to highlight the vein, blood samples were taken from the rat tail vein. Glucose oxidase-peroxidase reactive strips and a glucometer were used to estimate blood sugar levels.

Results

The goal of the current investigation was to determine whether an aqueous extract of *AS* leaves had any anti-diabetic effects on fasting blood glucose levels in albino rats that had been given STZ to induce diabetes.The study involved six groups of albino rats, each including six animals of either sex are weighing between 180-200g. For 15 days, the medication was taken once daily by mouth. *Amaranthusspinosus* impact on blood glucose levels in diabetic rats induced by STZ. The increase in blood glucose level in STZ-treated rats peaked on the fifth day and subsequently remained steady for the duration of the study.

According to the findings of the current investigation, a 15-day therapy with aqueous *Amaranthusspinosus* extract (200 mg/kg and 400 mg/kg) resulted in a highly significant (P 0.001) reduction in fasting blood glucose level. Blood glucose levels with 200mg/kg *AS* extract were 222.33±25.46 on day 10 and 157.02± 19.10 on day 15 treatment with aqueous extract of *AS* produced a significant (P < 0.001) decrease in fasting blood glucose level. And with 400 mg/kg of *ASLeaves* extract, the fasting blood glucose levels were 189.39 ± 18.65 on 10th day and 123.25 ± 3.063 on 15th day as compared to fasting blood glucose level of on 10th day 101.5±10.21 and 103.9±10.37 on 15thday in control animals.

Thus both 200mg/kg and 400 mg/kg of aqueous extract of *AS* showed dose dependent hypoglycemic effect by lowering the fasting blood glucose levels in comparison to the control group (Table& Graph). Effect of *Amaranthusspinosus* in Combination with Glibenclamide on Blood Glucose Levels in STZ–Induced Diabetic Rats slightly highly significant than the test doses i.e the fasting blood glucose levels were 162.66± 24.58 on 10th day and 103.58 ± 9.42 on 15th day of the study.

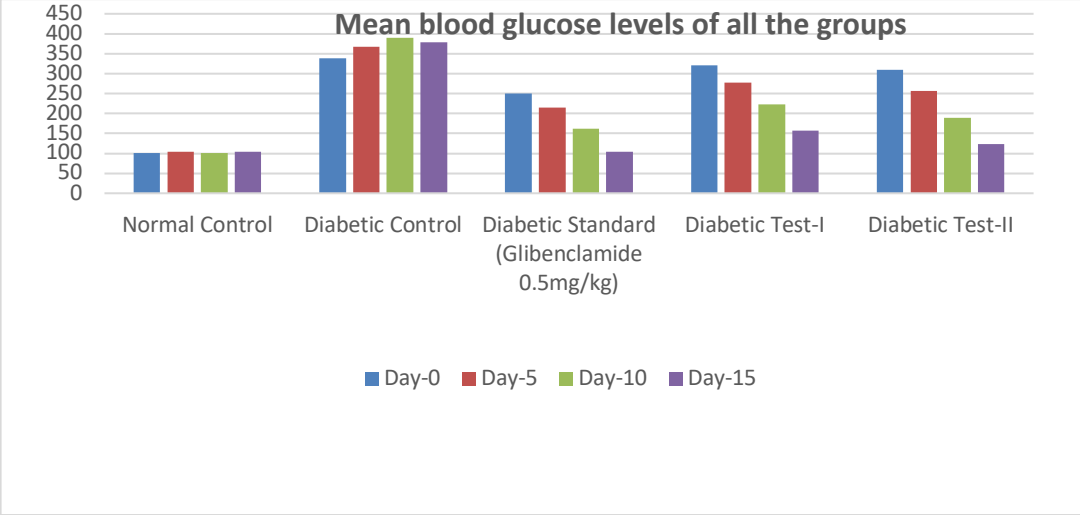

Figure 1

Table 1

Group	Day-0	Day-5	Day-10	Day-15
Normal Control	100.5±9.98	104.8±11.52	101.5 ±10.21	103.9±10.37
Diabetic Control	338.16±29.83	368.05±35.63	389.15±54.18	378.21±33.2
Diabetic Standard (Glibenclamide 0.5mg/kg)	250.16±20.58	215.08±24.54	162.66±24.58**	103.58±9.42***
Diabetic Test-I	321.17±17.29	277.65±12.49	222.33±25.46	157.02±19.10**
Diabetic Test-II	309.33±15.24	256.16±44.4	189.39±18.65*	123.25±30.63**

*All values are expressed as Mean ± SEM (n=6) $p<0.001$, $p<0.0001$ on 10^{th} day and 15^{th} day (ANOVA followed by Dunnet's multiple comparison test).

Discussion

Diabetes mellitus incidence is rising rapidly over the world, and we need supplementary medicines that are affordable. Despite the fact that insulin has grown to be one of the most significant therapeutic agents in medicine, efforts are still being made to identify synthetic or plant-based insulin alternatives, secretagogues, or sensitizers for the treatment of diabetes. Particularly in nations where access to the standard therapy of diabetes mellitus is insufficient, plants and their bioactive elements are utilised to treat diabetic mellitus. Numerous reports have been made regarding *Amaranthusspinosus* hypoglycemic behaviour.

In the present study, we investigate how an aqueous extract of *AS* (200 mg/kg and 400 mg/kg) affects fasting blood glucose levels in albino rats who have developed diabetes after being exposed to STZ. STZ is concentrated in the liver and islet cells after injection, where it is converted to dialuric acid. This acid undergoes oxidation back to STZ and produces O2, hydrogen peroxide, and hydrogen radicals when it is in aqueous solution. Superoxide dismutase (SOD), catalase, and glutathione peroxidase, which can scavenge these free radicals, are highly active in the liver. The islet cells, on the other hand, have low levels of these enzymes and are therefore susceptible to the cytotoxic effects of the free radical. According to reports, an increase in SOD activity in islet cells can either avoid or lessen STZ toxicity.

According to the findings of our investigation, rats that had been given STZ to induce diabetes had their fasting blood glucose levels dramatically (p0.001) reduced after receiving an oral aqueous leave extract of *AS* for 15 days. The blood glucose level was significantly (p0.001) reduced when AS extract was given and this effect was almost similar to that of glibenclamide alone.

The findings of the current investigation were consistent with the preceding research. At a dose of 200 mg/kg, a aqueous extract of the leaves of *AS* has been shown to have a notable hypoglycemic impact in both normal and streptozotocin-induced diabetic rats. It was believed that one of the mechanisms of insulin release was to stimulate beta cells [25].

Using mice that had been generated with diabetes by streptozotocin and normoglycemic glucose, the extract from *AS* leaves also shown anti-diabetic activity. In STZ-induced diabetic rats, the aqueous extract of AS (400 mg/kg) was reported to lower the levels of glucose, cholesterol, and triglycerides without having any toxic effects on the liver, as the biochemical indicators of liver damage, AST, ALP, and ALT, were observed in lower concentrations [26].

Recent research has revealed that the methanolic extract of AS leaves inhibits the DPPIV (dipeptidyl peptidase IV) and increases the GLP-1 (Glucagon Like Peptide) for type 2 DM, suggesting that it may be a promising candidate for further research and development as a potential anti-diabetic medication [27].The high total phenol and total flavonoid content of AS extract has been said to have strong antioxidant action. The reduction in diabetes sequelae shown in streptozotocin-induced diabetic rats may be attributable to these antioxidants' actions.

In a nutshell, *AS* extract improves cell repair and regeneration, boosts C peptide levels, increases peripheral glucose uptake, increases hepatic and muscle glycogen content, and protects cells from oxidative stress. By lowering glycated haemoglobin levels, restoring normal microalbuminuria, and modifying lipid profiles, it has an effect that is similar to insulin. Minimises long-term diabetes complications as a result.

As a result, the AS extract can be added as a neutraceutical or functional food to the natural diabetic treatments and may be safely, co-administered with an oral hypoglycemic medication for better glucose control, reducing the onset of diabetes mellitus problems.

Conclusion

The current study's findings demonstrate that *AS* extract has hypoglycemic activity, or the ability to lower blood sugar levels quickly, in STZ-induced diabetic rats. This effect is mediated by flavonoids, tannins, steroids, and trepenoids, and the aqueous extract of the leaves contains higher levels of phenols and flavonoids, which have greater

antioxidant activity, reducing the risk of diabetic complications.

Therefore, a combination of low dose oral hypoglycemic agent and low dose or even higher dose of *AS* leaves extract, which not only serves to control blood glucose levels but also has many advantages as mentioned, would be preferable to increasing the dose of the conventional oral hypoglycemic agent, which would result in more side effects. So above, can be used in the treatment of diabetes mellitus.

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