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Amaranthus spinosus L. (Amaranthaceae) leaf extract attenuates streptozotocin-nicotinamide induced diabetes and oxidative stress in albino rats: A histopathological analysis

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ABSTRACT

Objective: The aim of the present study was to evaluate the possible antidiabetic effects of Amaranthus spinosus leaf extract (ASEt) against streptozotocin-nicotinamide induced diabetes & oxidative stress in albino rats.**Methods:** Experimental diabetes was induced by a single dose of STZ (60 mg/kg) administered by intraperitoneal way after the administration of nicotinamide (120mg/kg). The oxidative stress was measured by reduced glutathione (GSH) content and by enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in liver and kidney. Biochemical observations were further substantiated with histological examination of pancreas, kidney and liver. **Results:** The increase in blood glucose with the decrease in GSH content and in enzymatic activities were the salient features observed in diabetic rats. Administration of ASEt (250 & 500 mg/kg bw/day, i.p) for 21 days caused a significant reduction in blood glucose in STZ-nicotinamide treated rats when compared with diabetic rats. Furthermore, diabetic rats treated with ASEt leaf extract showed a significant increase in the activities of both enzymatic and non-enzymatic antioxidants when compared to those of diabetic rats. Degenerative changes of pancreatic cells in STZ treated rats were minimized to near normal morphology by administration of ASEt leaf extract as evidenced by histopathological examination.**Conclusion:** Results clearly indicate that *Amaranthus spinosus* treatment attenuate hyperglycemia by decreasing oxidative stress and pancreatic cells damage which may be attributed to its antioxidative potential.

1. Introduction

Hyperglycemia evoked oxidative stress plays a crucial role in the development of diabetic complications, including nephropathy, neuropathy, retinopathy and hepatopathy, which is considered to result from augmented reactive oxygen species generation and decreased antioxidant defenses [1]. NIDDM is often associated with the most commonly occurring metabolic and physiologic problems, including elevated blood pressure, cardiovascular diseases, dyslipidemia and high cholesterol levels. Together with visceral obesity, this clustering of risk factors is known as the metabolic syndrome [2-3]. Plants are recognized as a wonderful source for medicines. It is estimated that 1200 species of plants are used as folk medicines for diabetes. *Amaranthus spinosus* (Amaranthaceae) is widely distributed throughout the tropics and warm temperate regions of Asia from Japan to Indonesia to India, 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 digestible, laxative, diuretic, stomachic, antipyretic, improves the appetite, biliousness, blood diseases, burning sensation, leprosy, bronchitis, piles and leucorrhoea.[4] Previous reports of this plant showed that its extract was used for its anti-inflammatory properties,[5] effect on hematology,[6] immunomodulatory activity,[7] anthelmintic properties,[8] antidiabetic, antihyperlipidemic and spermatogenic [9-10]. Chemically, it contains 7-p-coumaroyl apigenin 4-O-beta-D-glucopyranoside, a new coumaroyl flavone glycoside called spinoside, xylofuranosyl uracil, beta-D-ribofuranosyl adenine,

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betasitosterol glucoside, hydroxycinnamates, quercetin and kaempferol glycosides, betalains; betaxanthin, betacyanin amaranthine and isoamaranthine, gomphrenin, betanin, b-sitosterol, stigmasterol, linoleic acid, 0.15% rutin and beta-carotene.[11-12] The whole plants of *A. spinosus* are used for the treatment of diabetes in traditional system of medicine. Hence the present investigation is an endeavor to validate the scientific use of 50% ethanolic extract of leaves of *A. spinosus* (ASEt), against streptozotocin-nicotinamide induced diabetes in experimental animals.

2. Materials and methods

2.1. Plant material and extract preparation

The leaves of *A. spinosus* were collected from their natural habitat in and around Lucknow and authenticated by Taxonomist Dr. A. K. S. Rawat. The voucher specimen CIF-RB-2-126-1 was deposited in the departmental herbarium of National Botanical Research Institute Lucknow, India for future reference.

The freshly collected leaves (3 kg) were first air-dried and then dried in tray drier under control conditions and powdered. The powdered leaves (1200g) were macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 3L) by cold percolation method and centrifugation at 10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure and thus 125.0 g of solid residue (yield 12.5 % w/w) was obtained.

2.2. Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the identification of various active constituents by using standard procedure [13]

2.3. Drugs and chemicals

Streptozotocin (STZ) was purchased from Calbiochem USA. Nicotinamide was obtained from Ranbaxy Chemicals Ltd, India. All other chemicals and reagents used were of analytical grade.

2.4. Experimental animals

Healthy adult Wistar albino rats of both sex, aged between 2 and 3 months of age, weighing 200-250 g were used for the pharmacological studies. The animals were housed in polypropylene cages, maintained under standard conditions (12/12 h light and dark) at 25±3 ℃ and 35-60% humidity. They were fed with standard rat pellet diet (Amrut, India) and water ad libitum. The Institutional Animal Ethical Committee, United Institute of Pharmacy, Allahabad, India (No. 1451/PO/a/11/CPCSEA) has approved the study.

2.5. Acute oral toxicity study

The lethal median dose (LD50) determination was done in mice by OECD guidelines 423 [14]. A single dose of the extracts (5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg) in appropriate quantity of water was given orally by gavage to different group of mice (three each). The animals were allowed free access to water and food. However, all the animals were deprived of food for 2 hr before and 4 hr after dosing. The animals were continuously monitored during first 4 hrs and every one-hour during the first 12 hrs for any adverse effects. Later they were monitored (daily twice) for any abnormal changes throughout the study period (14 days). The extract was devoid of any toxicity in animals when given in dose up to 2000mg/kg. Hence for further study 250 & 500 mg/kg doses of extract were selected.

2.6. Experimental induction of diabetes

Streptozotocin (STZ) was freshly dissolved in (0.1M, pH 4.5) citrate buffer and Nicotinamide was dissolved in normal physiological saline and maintained on ice prior to use. All animals were allowed to adapt to cages for 3 days, after which they were fasted overnight. Non-insulin-dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of Streptozotocin (60 mg/kg b.w), 15 min after the intraperitoneal administration of nicotinamide (120 mg/kg b.w) all animals were given free access to food and water. Blood glucose levels were measured 2 days after STZ injection and used as parameters to obtain matching pairs of rats with diabetes of similar level of severity. Only rats with fasting blood glucose levels greater than 220 mg/dl were considered to be diabetic and were used in the experiment. The animals were randomly assigned to five different groups i.e. group I to V. Group I served as control containing 6 normal rats. All treatments started 3 days after STZ injection [15].

2.7. Experimental Design

Five groups of rats were used to study the effect of 50% ethanolic extract of *A. spinosus*.

Each group consists of six rats.

Group I - Control rats received vehicle normal saline solution

Group II - Diabetic control rats received vehicle normal saline solution

Group III and IV - Diabetic rats treated with extract 250 & 500 mg/kg body weight in respectively.

Group V- Diabetic rats treated with standard drug Glibenclamide 600 μg/kg body weight

The vehicles and the drugs were administered orally using