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ScienceDirect
European Journal of Integrative Medicine 2 (2010) 23–32
European Journal of INTEGRATIVE MEDICINE
www.elsevier.com/eujim

Original article

Antidiabetic and antihyperlipidemic activity of leaves of *Alstonia scholaris* Linn. R.Br.,

Sinnathambi Arulmozhi [a,*], Papiya Mitra Mazumder [b], Sathiyanarayanan Lohidasan [c], Prasad Thakurdesai [a]

[a] *Department of Pharmacology, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune 411 038, Maharashtra, India*
[b] *Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835 215, India*
[c] *Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Erandwane, Pune 411 038, Maharashtra, India*

Received 25 August 2009; received in revised form 29 November 2009; accepted 9 December 2009

Abstract

Aim of the study: Alstonia scholaris Linn. (R.Br.,) has been used in traditional and folklore medicine for the treatment of diabetes. The aim of the present study was to evaluate the effect of ethanolic extract of the leaves of *A. scholaris* (known as EEAS) in streptozotocin-induced diabetic rats.

Materials and methods: The streptozotocin-induced diabetic rats were orally treated with vehicle (2% w/v Tween 80), glibenclamide (0.25 mg/kg) and EEAS (100, 200 and 400 mg/kg) to the respective treatment groups. The blood glucose level, body weight, glycosylated hemoglobin, muscle and liver glycogen, lipid profile, lipid peroxidation, antioxidant status were measured and histopathology of pancreas was performed after 6 weeks of treatment and compared to the control.

Results: EEAS and glibenclamide were found to significantly ($p < 0.001$) reduce the blood glucose level, glycosylated hemoglobin and lipid peroxidation, whereas they increased body weight, liver and muscle glycogen and antioxidant status. The antidiabetic effect was sustained from 1 week onwards till the end of the study. The histopathology of pancreas revealed that the pancreatic β-cell damage with streptozotocin did not reverse in any of the treatment groups.

Conclusion: It has been concluded that EEAS, in addition to the antidiabetic activity, also possess antihyperlipidemic and antioxidant activities in the streptozotocin-induced diabetic model.

Keywords: Antidiabetic; Antihyperlipidemic; *Alstonia scholaris*; Antioxidant

Introduction

Alstonia scholaris Linn.R.Br., belongs to the family of Apocynaceae and is native of India. It is growing wild throughout in indeciduous, evergreen forests and even in plains. Bark of *A. scholaris* possess a spectrum of pharmacological activity, ranging from bitter, astringent, thermogenic, laxative, antipyretic, anthelmintic to galactogoguic and cardiotonic properties; therefore, used in fever, malarial fever, abdominal disorder, dyspepsia, leprosy, skin diseases, asthma, bronchitis, cardiopathy, etc. [1,2]. An antimalarial Ayurvedic preparation, Ayush-64, containing *A. scholaris* is marketed [3]. Folklore use includes application of milky juice of leaves on wounds, ulcers and for rheumatic pain, as well as a form mixed with oil which is applied for earache [1]. Extract of *A. scholaris* is reported to possess several pharmacological activities that include antiplasmodial activity [4], antimutagenic effect [5], immunostimulatory effect [6] and hepatoprotective activity [7]. Echitamine, an indole alkaloid extracted from the bark was found to exhibit anticancer activity [8,9].

Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. In recent times, focus on plant research has increased throughout the world and the immense potential of medicinal plants used in various traditional systems has been established scientifically. Many plant products are used widely in folklore medicine

* Corresponding author. Tel.: +91 9371085077.
E-mail address: pharmarul@gmail.com (S. Arulmozhi).

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doi:10.1016/j.eujim.2009.12.001

because of their therapeutic potential. It is widely accepted that folk or traditional medicinal uses of plants indicate the presence of biologically active constituent(s) in a plant. Screening plants with such ethnomedical uses is believed to increase the odds in discovering new medicines [10]. The leaves of *A. scholaris* Linn. R.Br., are reported to be used in diabetes in folklore and traditional medicine [11] and also reported to have *in vitro* α-glucosidase inhibitor activity [12]. Based on the above perspective, an effort was made to ascertain the possible role of EEAS in streptozotocin-induced diabetes mellitus *in vivo*.

Materials and methods

Collection and authentication of plant

The leaves of *A. scholaris* (Family: Apocynaceae) were collected in the months of September–October 2008 from the hills of Sawantwadi, Maharashtra, India. The plant material was taxonomically identified by Dr. P.S.N. Rao, Botanical Survey of India (BSI), Pune and the voucher specimen AS-1 is retained in the herbarium of BSI, Pune for future reference.

Preparation of ethanolic extract of leaves of A. scholaris

The dried powdered leaves (500 g) were defatted using petroleum ether and subjected to extraction in a Soxhlet apparatus by using ethanol. The solvent was removed from the extract under reduced pressure to obtain a semisolid mass and was vacuum dried to yield solid residue (5.24% w/w ethanol extract). The extract (EEAS) showed positive tests for alkaloids, tannins, saponins, glycosides, triterpenoids and flavonoids.

Chemicals and reagents

Streptozotocin (Sigma chemical co., U.S.A), glibenclamide (Prudence Pharma Chem, India), glucose estimation kit (Span diagnostics, India), triglyceride estimation kit (Span diagnostics, India), HDL estimation kit (Span diagnostics, India), total cholesterol estimation kit (Span diagnostics, India) were used. Other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Animals

Albino Wistar rats of either sex weighing between 150 and 180 g were used for the present study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee (Reg. no. 100/1999/CPCSEA), Approval no. CPCSEA/44/2008. CPCSEA guidelines were adhered during the maintenance and experiment.

Acute toxicity study

Acute toxicity study was carried out for the EEAS following OECD guidelines [13]. The extract suspended in water with 2% w/v Tween 80 in the dose of 2 g/kg body weight was orally administered to overnight-fasted, healthy rats ($n = 3$). The animals were observed continuously for 24 h for mortality.

Induction of experimental diabetes mellitus and treatment protocol

The animals were divided into six groups of six animals each as follows:

Group I—vehicle control, 2% w/v Tween 80, p.o. (non-diabetic)
Group II—diabetic control
Group III—diabetic standard treated, 0.25 mg/kg of glibenclamide, p.o.
Group IV—diabetic EEAS 100 mg/kg, p.o.
Group V—diabetic EEAS 200 mg/kg, p.o.
Group VI—diabetic EEAS 400 mg/kg, p.o.

Diabetes was induced in all groups except vehicle control following overnight fasting (deprived of food for 16 h allowed free access to water) by a single intraperitoneal injection of 65 mg/kg of streptozotocin (STZ) dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5). The animals of vehicle control (Group I) were injected with buffer alone. Streptozotocin-injected animals were given 5% glucose solution (2 ml/kg body weight) for 24 h following streptozotocin injection to prevent initial drug-induced hypoglycemic mortality. After 72 h, blood was withdrawn by retroorbital puncture under light ether anesthesia and the blood glucose level was estimated. After 1 week of induction, blood glucose level was estimated again and a fasting blood glucose level of more than 200 mg/dL was considered as diabetic [14]. The treatment was orally given to the respective groups once a day for 6 weeks.

Estimation of plasma glucose, body weight and lipid profile

Every week, following overnight fasting (16 h fasting with free access to water), the blood samples were withdrawn from the animals by retroorbital puncture under light ether anesthesia. The plasma glucose estimation was done by the glucose oxidase/peroxidase (GOD/POD) [15] method using a standard kit obtained from Span Diagnostics, India. Body weight of all experimental animals was recorded using a digital weighing scale. The serum triglycerides (TG), total cholesterol (TC) and high-density lipoproteins (HDL) levels were estimated [16] using standard kits obtained from Span Diagnostics, India. Serum low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) levels were calculated by using the following formula:

$$\mathrm{VLDL} = \mathrm{TG}/5$$

$$\mathrm{LDL} = \mathrm{TC} - (\mathrm{HDL} + \mathrm{VLDL})$$