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Andrographolide: A Renoprotective Diterpene from *Andrographis Paniculata (Burm. f.) Nees*

P. Singh[1], M. M. Srivastava[1], D. K. Hazra[2] and L. D. Khemani[1]
[1]Department of Chemistry, Dayalbagh Educational Institute (Deemed University), Agra, India.
[2]Nuclear Medicine Unit, S. N. Medical College, Agra, India.
Email: singh.prati@gmail.com

Abstract

Andrographis paniculata Nees, a well-known plant of Indian and Chinese traditional system of medicines, has drawn attention of researchers in recent times. An attempt was made to isolate the active principle from the plant using chromatographic methods. Andrographolide isolated from Andrographis paniculata (burm. f.) Nees (Acanthaceae) ameliorated the diabetes induced renal failure when treated for 28 days. The results demonstrated that andrographolide showed alleviation in terms of serum creatinine (54.73%), serum urea (63.92%), urinary proteins (32.35%) at a dose of 90 μg/ml. The structure of the isolated compound was confirmed by various spectroscopic methods.

Introduction

Diabetic nephropathy is a common problem that is most likely to occur in patients who have worse glycemic control, hypertension, glomerular hyperfiltration or a genetic predisposition. The lifetime risk of nephropathy is roughly equivalent in type 1 and type 2 diabetes [1]. Diabetic nephropathy is one of the major complications of non-insulin dependent diabetes mellitus (NIDDM) which is a common cause of death in diabetic patients. The severity of renal disease in diabetic patients correlates with the levels of blood urea and serum creatinine [2]. Diabetic nephropathy accounts for considerably morbidity and mortality even in patients with well controlled blood sugar values [3]. Insulin therapy and oral hypoglycemic agents offer effective glycemic control; yet, their shortcomings limit their usage [4].

Plants are reputed in the indigenous systems of medicine for the treatment of various diseases [5]; the available literature shows that there are more than 800 plant species showing hypoglycemic activity [6]. The world health organization has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs [7]. Phytochemicals isolated from plant sources are used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure [8].

The hunt for complementary and alternative medicine is an ongoing process in the area of renal failure research. *Andrographis paniculata (Burm. f.) Nees* (Acanthaceae), a renowned plant in South-Asian traditional medicine is an established antidiabetic herb; hence attracts attention towards exploring the possible anti renal failure properties, especially against diabetic nephropathy. Andrographis paniculata is reported as a cold property herb in TCM and is used to get rid of body heat and to expel toxins. The plant is particularly known for its extremely bitter properties (often called king of bitters) and is used traditionally as a remedy against common cold, dysentery, fever, tonsillitis, diarrhea, liver diseases, inflammation, herpes, etc. [9–11]. The traditional uses and pharmacological aspects of A. paniculata have been well-documented in an extensive review recently [12]. A number of active principles are reported from the plant, which mainly include diterpene lactones, flavonoids and polyphenols [13–14]. However, the prime constituent andrographolide has been is mainly attributed for its therapeutic properties. Diterpenoid lactone andrographolide ($C_{20}H_{30}O_5$) is the principle compound found in A. paniculata, which is mainly concentrated in leaves and can be easily iso-

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lated from the crude plant extracts as crystalline solid [15–16]. The structure of the compound has been elucidated by X-Ray crystallographic analysis and the molecular stereochemistry, bond distances, bond angles, etc. all were determined [17]. Chemically designated as (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl) -5, 8-adimethyl-2-methylene-1-napthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone),andrographolide (Fig. 1) exhibits extraordinarily vast range of biological activities [18–23]. In recent past, the compound is reported for its anti-tumor, anti-HIV and Cardioprotective properties [24–30]. In view of present literature and our previous experiments [31–32], an attempt was made to evaluate the effectiveness of isolated active principle 'Andrographolide' from *Andrographis paniculata* against stz-induced diabetes and renal damage in rats by studying the effect on blood urea and serum creatinine and urinary proteins levels.

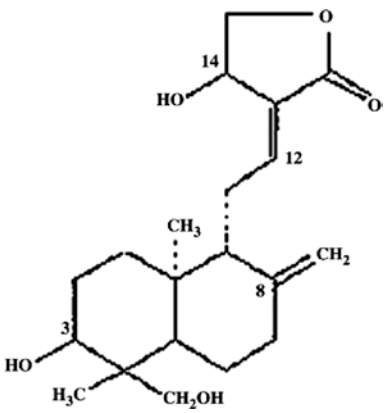

Fig. 1: Structure of the principle phytochemical compound of andrographolide

Materials and Methods

Plant Material

Plants were procured from NBRI, Lucknow (Accession No. AP-D02, Herbarium Voucher No. 445785) for the study. The fresh plants were washed quickly with the water to remove any foreign matter, shade dried and powdered and stored in airtight containers.

Isolation of Andrographolide

Andrographis paniculata (300 g) were macerated and extracted with methanol. The solvent was concentrated *in vacuo* to yield methanol extract (30 g), which was diluted with aqua distillate, and then partitioned by ethyl acetate, from which 10 g residue was obtained. The ethyl acetic fraction was subjected to silica column chromatography and gradiently eluted with chloroform methanol to afford andrographolide. The andrographolide crystal was recrystalized from hot methanol. Its IR, ^{1}H-NMR, ^{13}C-NMR and MS data were in accordance with those of andrographolide [33] and standard (Sigma, USA). Isolated andrographolide equivalent to dose 90 µg/ml was administered to rats.

Animals

Male Wistar albino rats weighing 250±50 g were housed in polypropylene cages and maintained at 24 ± 2 °C under 12 hour light/dark and 60 ± 5 % humidity. They were fed with Amrut Laboratory Animal Feed, manufactured by Nav Maharashtra Chakan Oil Mills Ltd., Pune, India. Water was provided *ad libitum*. The animals were acclimatized for a week under laboratory conditions. All experiments were performed according to the norms of the local ethical committee.

Experimental Design

Experimental animals were distributed randomly, in eight groups, containing six animals each.

a. Normal animals

Group I received vehicle only, throughout the experimental period.

b. Streptozotocin induced diabetic animals

Diabetes was induced in rats deprived of water for 24 hr followed by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) [34] dissolved in freshly prepared 0.01 M sodium citrate buffer, pH 4.5 [35] and the rats were left to develop diabetic nephropathy for a week. A week after the STZ injection, blood glucose levels were determined in blood samples collected from the tail vein, using Glucometer (Accu-Chek, one touch ultra). Animals were considered to be diabetic if they had elevated plasma glucose

concentrations > 250 mg/dl [36]. Then, these diabetic rats, referred to as STZ rats, were considered to show diabetic nephropathy if their blood urea and creatinine values were elevated. These animals further divided into two groups. Group II received vehicle only and group III received andrographolide, daily at a dose of 90 µg/ml (p. o.), respectively, for 28 consecutive days. Renoprotective activity against streptozotocin induced diabetic nephropathy, was screened in terms of alleviation in serum creatinine and serum urea.

Sample Collection

Individual rats belonging to different groups were placed in metabolic cages over a period of 24 h and urine was collected. At the end of 24 hours, rats were anesthetized with a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood samples were collected via retro orbital puncture in plain plastic tubes, left to stand at 4 °C for 1 hour, and centrifuged (900 × g for 15 min at 5 °C) to separate serum. The serum obtained was stored at – 5 °C until analysis.

Biochemical Analysis

Plasma and urine samples were assayed using standard diagnostic kits, viz. serum creatinine (Human, Germany), serum urea (Beacon Diagnostics, India) and urinary protein (ERBA Diagnostics, Germany).

Statistical Analysis

All values were expressed as mean ± standard error. Differences within groups were evaluated by paired *t*-test. One-way analysis of variance was used for examining differences among groups. Inter-group comparisons were made with Dunnet's multiple-comparison test. A p-value of < 0.05 was considered to indicate significance.

Results and Discussion

A. paniculata is cited in Ayurveda as a plant with diuretic, antioxidant and antidiabetic properties. Our previous study indicating renoprotective activity of *Andrographis paniculata* in gentamicin induced acute renal failure, thereby suggests this study as a complementary research for its anti renal failure effects against streptozotocin induced diabetic nephropathy.

Andrographolide (Figure 1) showed a significant alleviations in diabetes induced renal failure. Data presented in table 1 demonstrate that the isolated compound 'andrographolide' showed amelioration in terms of serum creatinine (54.73 %), serum urea (63.92 %), and urinary proteins (32.35 %) at a dose of 90 µg/ml. The isolated compound also diminished the blood glucose level. The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs [36–38]. Diabetes mellitus of long duration is associated with several complications such as atherosclerosis, myocardial infarction, nephropathy etc. These complications have long been assumed to be related to chronically elevated glucose level in blood [39].

Table 1: Change in renal profile after the treatment of diabetic nephropathy rats with andrographolide at a dose of 90 µg/ml

Groups/Biomarkers	Andrographolide	
	0^{th} Day	28^{th} Day
Serum		
Glucose (mg/dl)	314.22 ± 0.12	112.14 ± 0.44 (–64.31)
Creatinine (mg/dl)	2.43 ± 0.11	1.10 ± 0.02 (–54.73)
Urea (mg/dl)	89.02 ± 3.21	32.12 ± 1.14 (–63.92)
Urine		
Proteins (mg/dl/day)	10.54 ± 1.56	7.13 ± 0.08 (–32.35)

Values are mean ± SE of 6 rats.
Figures in parenthesis indicate percent change with respect to 0 day.

$p<0.0001$ (28^{th} day is compared with 0^{th} day).

Renal disease is one of the most common and severe complications of diabetes. In diabetes mellitus, increased blood glucose, lipids and oxygen free radicals can induce glomerulosclerosis and chronic tubulointerstitial damage in the kidneys leading to diabetic

nephropathy [39–43]. A progressive decline in the glomerular filtration rate due to loss of functioning nephrons and histological renal damage are common characteristics in the development of diabetic nephropathy [44].

Seven days after streptozotocin injection, the serum urea concentrations significantly elevated ($p<0.01$) in STZ induced diabetic nephropathy models. There were significant elevations in serum creatinine and urea levels when compared to that of the normal control animals, indicating impaired renal function of diabetic animals. In renal disease, the serum urea accumulates resulting in uremia, as the rate of urea production exceeds the rate of clearance [45]. Urea is the principal end product of protein catabolism and accumulates with renal failure. *A. paniculata* treatment showed a significant amelioration in all the renal biomarkers evaluated in the study, in a solvent-dependent manner. These results indicate that propolis can attenuate renal damage in diabetic rats. *A. paniculata* has a strong antioxidant and free radical scavenging effect [46]. This finding suggests that *A. paniculata* may improve the disturbed metabolism associated with diabetes. In this study, serum levels of glucose, was significantly elevated in diabetic rats compared to that of the normal control rats.

The present piece of work demonstrates that MeOH extract of the plant *A. paniculata* exhibited renoprotective activity against diabetic nephropathy. The chromatographic fractionation of the MeOH extract to its derived compound a diterpene lactone 'Andrographolide' has resulted into the amelioration with an effective concentration of 90 µg/ml and hence makes the plant metabolite interesting for further research.

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