Hypoglycemic Response of *Andrographis paniculata* and andrographolide

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Abstract

The aim of this study is to evaluate hypoglycemic properties of *Andrographis paniculata* (*A. paniculata*) Ethanolic exract of *A. paniculata* and its active constituent andrographolide offers a therapeutic value in prevention of diabetes.

The hypoglycemic effects could be mainly attributed to its antioxidant properties which prevent liver damage. The significant changes observed in lipid per oxidation along with enhancement of antioxidant defense system. The constituent present in *A. paniculata* enhance the antioxidant properties which create hypoglycemic properties showing lowering in serum glucose, enhance body weight, increase serum protein and acute alteration in serum cholesterol and blood urea. Further studies will be needed in future to determine the main active ingredients having the beneficial antidiabetic and antioxidant effect.

Keywords: *Andrographis paniculata*, diabetes, andrographolide

Introduction

Over the past decade, herbal medicine has more relevance in health care with repercussion for both global health and trade. *Andrographis paniculata* (Burm, F.) Nees, is an herbaceous plant belong to the family acanthaceae and is found throughout tropical and sub-tropical Asia and India. It is commonly known as Kalmegh and Kings of bitters. Extracts of this plant and andrographolide exhibit pharmacological activities such as hypoglycemic, antibacterial and antiviral. A major active constituent, andrographolide exhibits a broad range of biological activities such as anti-inflammatory, antitumor, anti-diabetic, antimalerial and hepatoprotective[1]. In recent decades, numerous andrographolide derivatives have been discovered and used for cure various health disorder.

Diabetes mellitus is one of the main threats to human health in 21st century. Since type-2 diabetes accounts 90% of all cases worldwide, the current diabetes epidemics is attributed predominantly to rising cases of type-2 diabetes. Type-2 diabetes is connected to oxidative stress due to hyperglycemia and hyperlipidemia which induce inflammatory-immune responses and oxidative stress reactions, therefore the generation of free radicals accounts for aggravation of type-2 diabetes

and cardiovascular complications[2]. Glucosidase catalyses the final step of carbohydrate metabolism in biological system. Inhibition of this enzyme is one of the ways to decrease postprandial blood sugar increase and damage cause in type-2 diabetes. *A. paniculata* phenolics constituents are known for their α-glucosidase inhibitory potential and when combined with their antioxidant activity could be relevant in early stage management of type-2 diabetes[3]. *A. paniculata* have been reported as having antibacterial, antifungal antiviral, choleretic, hypoglycemic and adaptogenic effect. Four lactones chuanxinlian A (deoxyandrograpnolide), B (andrograpnolide), C (neoandrographolide) and D (14-deoxy-11,12 dihydroandrograpnolide) were isolated from aerial parts[4].

Cytochrome P-450 enzymes are known as a super family of haemoprotein. This enzyme is mainly responsible for hepatic function as well as type-2 diabetes[5,6]. Hepatocelular damage may be result from chromic metabolizing of xenobiotics including drugs, toxins and chemical carcinogens in the liver. Cytochrome 450 is antihepatotoxic enzymes pyruvate transaminase (GPT), glutamate oxalo acetate transminase (GOT), alkaline phosphatase (ALP) and acid phosphate (ACP). Phase-I and phase-II biotransformation enzymes are involved in the metabiotic activation and detoxification of various toxins. Phase-I enzymes convert xenobiotics to activate intermediates and phase-II enzymes catalyze the conjugation of these active intermediates with endogenous cofactors to increase their water solubility and facilitate their excretion through urine arbiter[7,8]. These study indicates that for hypoglycemic active analysis of blood glucose, body weight, serum cholesterol, blood urea, serum protein with reveal new result for antidiabetic activity of *A. paniculata* and its active constituents[9].

Material & Methods

Plant material- *A. paniculata* were collected from rood side of Jaunpur district U.P. India. The plants was authenticated by herbarium of botany department, Kutir P.G. College, Chakkey, Jaunpur under guidance of Prof. R.A. Singh Department of Taxonomy.

Extraction of plant and isolation of andrograpnolids- The *A. paniculata* whole plant powdered 260g was defatted with petroleum ether (40:60) and then extracted with ethyl alcohol by using

Soxhlet apparathus. The solvent was removed by distillation. Semisolid extract proceed for cloumn chromatography for andrographolide extraction. Extracted compound purified by recrystallization and identified by co-TLC and spectroscopic analysis.

Animal

The experiment was carried out on sures albino mice of both sexes, weighing between 35 to 40 gm. They were procurred from animal house, Institute of Medical Sciences, BHU, Varanasi (U.P.). The animals were acdimatized to the standard laboratory conditions in cross ventilated animal house at temperature 30±2ºC relative humidity 50-62% and light and dark cycles of 12:12 hrs, fed with standard pallet.

For this experiment, a total 36 mice were used. Mice were divided in six groups comprising 6 animals in each group as under:

Group-1: Serves as control and received orally 0.5% carboxy methyl cellulose (3ml each) upto 30 days.

Group-2: Serves as diabetic control.

Group-3: Receive oral dose of 200 mg/kg bw. *A. paniculata* extract respectively for 30 days.

Group-4: Receive oral dose of 400 mg/kg bw. *A. paniculata* extract respectively for 30 days.

Group-5: Receive oral dose of Andrographolid 5 mg/kg bw. for 30 days.

Group-6: Serves as received oral dose of standard drug Glibenclamide (10 mg/kg) bw for 30 days.

Streptozotocin at a dose of 40 gm/kg body weight I.P. were administer in all animals in the group of II, III, IV, V and VI.

Sample collection

At the end of experiment 30 days, animal anaesthetized by using ketamine (25 mg/kg bw) intra muscular injection. Blood was collected from retro orbital puncture after 12hrs fasting and 2hrs giving the extract in gun acacia for the estimation of plasma glucose. Auto analyser and Kit

methods were used in this study for glucose estimation. Blood was collected in tubes with EDTA for the estimation of plasma lipid and peroxide marker and antioxidants.

Analytical Methods

Determination of fasting serum glucose

Fasting serum glucose was estimated by using commercially available glucose oxidase-peroxidase reactive test strips and measured on a portable blood glucose meter (Accu-Chek® Advantage-II glucose meter, Roche Diagnostics, Germany).

Determination of serum cholesterol, blood urea and serum protein

Serum cholesterol, urea and protein were estimated by the method of Kim and Goldberg[10]. Serum triglyceride and free fatty acids were analyzed by the methods of Soloni[11] and Soloni and Sardina[12] respectively.

Results and Discussion

Diabetes mellitus is an metabolic disorder characterized with hyperglycemia and free radical production. Experimental medicine glibendamids have not satisfactory effective therapy is till available to cure diabetes millitus. In diabetes mellitus condition, protein glycation and glucose autoxidation may produce free radicals which in turn catalyze lipid peroxidation[13,14]. Our results reword that ethanol extract of *Andrographis paniculata* (400 mg/kg of body weight) reduced the blood glucose and free radicals levels in diabetic was when compared to normal rats. Table-1 shows the levels of blood glucose increased in diabetic rats as compared to normal. The treatment of oral administration of *A. paniculata* ethanol extract and andrographolide in diabetic rats significantly decreased and brings back to near normal level when compared to diabetic rats. *A. paniculata* ethanol extract when compared to 200 mg/kg, the 400 mg/kg of body weight and andrographolide 5 mg/kg bw have the highly significantly ($P<0.01$) reduce the blood glucose level.

Table-1 : Effect of *A. paniculata* ethanolic extract and andrographolide on blood glucose in normal and experimental diabetic rats.

Groups	Blood glucose in Initial (mg/dl)	Blood glucose (mg/dl) in 32nd day
Normal	79.1 ± 0.03	82.40 ± 0.9
Diabetes	215.5 ± 0.04	226.4 ± 0.11*
A. paniculata ethanolic extract (200 mg/kg)	212.8 ± 0.5	101.7 ± 0.2**
A. paniculata ethanolic extract (400 mg/kg)	221.4 ± 1.2	106.6 ± 1.0**
Andrographolide (5 mg/kg bw)	221.6 ± 0.4	102 ±0.0**
Glibenclamide (10 mg/kg body wt.)	220.2 ± 0.3	91.4 ± 0.0**

Values are expressed as mean ± Sd for six animals in each group;
*P<0.05 and **P<0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

During the experimental periods the changes in body weight were observed. The body weight found to be increased in normal and *A. paniculata* plant ethanol extract and andrographolide of treated diabetic rats and in diabetic rats it was decreased when compared with normal Table-2.

Table-2 : Effect of *A. paniculata* ethanolic extract and andrographolide on animal body weight in normal and experimental diabetic rats.

Groups	Initial body weight (g)	Body weight (g) on 30th day
Normal	181.3 ± 0.3	193.60 ± 0.2
Diabetes	179.4 ± 0.4	142.7 ± 0.3*
A. paniculata ethanolic extract (200 mg/kg)	183.8 ± 0.4	189.6 ± 0.2**
A. paniculata ethanolic extract (400 mg/kg)	182.6 ± 1.1	191.6 ± 0.1**
Andrographolide (5 mg/kg bw)	180.0 ± 0.03	194 ± 0.2**
Glibenclamide (10 mg/kg body wt.)	183.3 ± 0.03	193.7 ± 0.2**

Values are expressed as mean ± Sd for six animals in each group;
*P<0.05 and **P<0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

Administration of *A. paniculata* ethanol extract and andrographolide in diabetic rats exhibited considerable gain of body weight. During steady state treatment, when the rats administered *A. paniculata* ethanol extract and andrographolide had improved their glucose homeostasis to some extent, their food intake was similar and their growth rate was parallel. It suggests that *A. paniculata* extract and andrographolide has the anabolic action after initial adaptation to the treatment[15]. Normalization of blood glucose by *A. paniculata* ethanol extract and its active compound at 400 mg/kg for 30 days treatment may be due to enhanced glucose transport across the cell membranes and

glycogen synthesis and or glycolysis same result observed with andrographolide treatment. In this context thus are many number of other plants have been observed to have hypoglycaemic effect[16,17].

The changes in urine sugar during the experimental period were observed to find out the effectiveness of the ethanolic extract of *A. paniculata* and andrographolide. The excretion of urine sugar was found to be increased in diabetic rats when compared with normal *A. paniculata* plants ethanol extract and andrographolide treated diabetic rats was reverses back to the significantly normal condition, the absence of urine sugar in experimental rats[18,19]. *A. paniculata* ethanol extract and andrographolide when compared 200 mg/kg of body weight, the 400 mg/kg of body weight and 5 mg/kg body weight of andrographolide have the highly significantly ($P<0.01$) increased the body weight and decreased urine sugar level experimental rats. Table-3 shows the effect of *A. paniculata* extract and andrographolide on serum protein in normal, diabetic *A. paniculata* extract and andrographolide treated diabetic rats. It was found that the level of serum protein was found to be decreased in streptozotocin induced diabetic rats when compared with normal. The decreased level of serum rats when compared with normal. The decreased level of serum protein was bringing back to significantly ($P<0.01$) near normal in *A. paniculata* ethanol extract treated experimental diabetic rats.

Table-3 : Effect of *Andrographis paniculata* ethanolic extract on serum protein in normal and experimental diabetic rats.

Parameters	**Normal**	**Diabetes**	***A. paniculata* ethanolic extract (200 mg/kg)**	***A. paniculata* ethanolic extract (400 mg/kg)**	**Glibenclamide**	**Andrographolide 5 mg/kg bw**
Serum protein (g/dl)	6.9±0.22	6.1±0.05**	6.7±0.23*	6.8±1.32**	6.8±0.41**	8.9±0.38

Values are expressed as mean ± Sd for six animals in each group;
*P<0.05 and **P<0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

In diabetic rats, serum protein was decreased[20]. Dehydration and loss of body weight have been associated with diabetic rats. The decreased protein indicates the polyphagia condition and loss of body weight associated due to excessive breakdown of tissue protein and protein wasting due to unavailability of carbohydrates as an energy source[21]. After the treatment of *A. paniculata* ethanol extracts and active compound andrographolide these was significant improvement in the plasma protein.

The levels of serum cholesterol and blood urea were found to be elevated in streptozotocin induced diabetic rats when compared to normal rats. After treatment of *A. paniculata* extracts, it brings back to near normal levels of cholesterol and blood urea in experimental rats (Table-4). The 400 mg/kg of body weight *A. paniculata* was significantly decreased in serum cholesterol and blood urea level when compared to 200 mg/kg of body weight.

Table-4 : Effect of *Andrographis paniculata* ethanolic extract on cholesterol and blood urea in normal and experimental diabetic rats.

Parameters	**Normal**	**Diabetes**	***A. paniculata* ethanolic extract (200 mg/kg)**	***A. paniculata* ethanolic extract (400 mg/kg)**	**Glibenclamide**	**Andrographolide 5 mg/kg bw**
Serum cholesteroal (mg/dl)	161.0±1.40	243.4±0.23*	175.3±0.22*	171.4±0.23**	166.3±1.31**	169±0.23**
Blood urea (mg/dl)	25.50±1.04	48.03±1.22*	29.35±0.22*	27.23±1.04**	26.40±1.21**	26±0.52**

Values are expressed as mean ± Sd for six animals in each group;
*P<0.05 and **P<0.01 significantly compared with diabetes Vs normal and diabetes Vs treatments.

In streptozotocin induced diabetic rats, the concentration of urea in liver was doubled when compared to normal. The increase may be due to the enhanced catabolism of both liver and plasma proteins. Almdal *et al.* [22], have reported that insulin therapy in diabetes leads to normalization of organ nitrogen contents of urea synthesis. The cholesterol level of diabetic rats was also increased by two fold. The de nova synthesis of cholesterol in the gut of diabetic rats in increased two fold when compared to other organs of the systems and this may be the reason for the increase in serum cholesterol level in diabetic rats.

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