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Research article

Preclinical evaluation of standardized anti-diabetic herbal formulation (ADC-05)

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Key words: Diabetes, herbs, glucose, antioxidant, α-amylase, α-glucosidase.

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Abstract

ADC-05 a new formulation was prepared with nine standardized herbal extracts for anti-diabetic actions to find out the anti-diabetic potentialities in experimental animals as well as explore its potential mode of actions. ADC-05 exhibited rich in phenolics and showed DPPH antioxidant scavenging actions. Moreover, it showed α-amylase and α-glucosidase inhibition dose dependently. ADC-05 did not showed any signs of toxicity or mortality up to 2.0 g/kg per oral dose in mice. Oral administration of ADC-05 at the dose of 100 mg/kg and 200 mg/kg significantly declined the fasting blood sugar level within 2 h and 14 days consecutive treatments, similar to Glibenclamide in Streptozotocin induced hyperglycemic rats. In conclusion it may infer that new formulation ADC-05 has antidiabetic potentialities in rats.

Introduction

Diabetes is fast gaining the status of a potential epidemic capital in India with more than 62 million diabetic individuals at present diagnosed with the disease [1]. Geographical and ethical influences have exposed that people of Indian origin are highly prone to diabetes [2]. Currently available therapies include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides, but not pleasing to control its complications [3]. Prior to development of insulin injection therapy in 1921 diabetes was entirely managed with herbal medicine. More than 1200 species of organisms have been used ethno-pharmacological reports to treat symptoms of diabetes. The major chemical constituents of plants credited to anti-diabetic action are glycosides, alkaloids, glycans, triterpenes, mucilages, polysaccharides, oils, vitamins, saponins, glycoproteins, peptides, amino acids and proteins [4-5]. Indian traditional remedies for diabetes are usually mixed formulations containing blood sugar lowering herbs in combination with immune modulators, hypocholesteremic, antioxidants, diuretics and de toxicants [6-7]. The herbal drugs clinically used to treat diabetes can be mainly divided into insulin secretagogues, insulin sensitivity improvement factors, insulin-like growth factor, aldose reductase inhibitor, α-glucosidase inhibitors, and protein glycation inhibitor, almost all of which are chemical and biochemical drugs [8].

Clinical trial reported that seed powder of *Syzygium cumini* has blood glucose lowering action in diabetic patients [9]. Mitra and Bhattacharya (2006) reported that *Trigonella foenum graecum* (fenugreek) powder has significant anti-diabetic and dislipidaemic potentiality in diabetic patients [10]. *Azadirachta indica* is a wonder plant and perhaps the most useful traditional medicinal plant in India including diabetes [11]. Fruit extract of *Emblica officinalis* has significant anti-diabetic and hypolipidemic activities [12]. Gupta *et al.,* (2010) reported the anti-diabetic activity of aqueous leaf extract of *Cassia auriculata* [13]. *Gymnema sylvestre* leaf extracts showed insulin secretagogues action [14]. *Andrographis paniculata* has been appreciable for α-glucosidase inhibitory actions while, *Tribulus terrestris* has both α-glucosidase and aldose reductase inhibitory actions [15-16]. ICMR group reported that *Pterocarpus marsupium* has an effective blood glucose lowering action with type 2 diabtes patients and free from any significant side effects [17]. On the basis of these information, ADC-05 has been formulated with standardized 50% hydroethanolic powdered extracts of the above mentioned herbs (Table 1). The objective of the present study was to find out the anti-diabetic potentialities of ADC-05 in experimental animals and also explore its potential mode of actions. This is the first report of ADC-05 on anti-diabetic properties.

Experimental

Test drug preparation

Standardized dry 50% ethanolic powdered extract of nine ingredients of ADC-05 was weighing proportionately and dissolved in required volume of deionized water as given in Table 1.

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Table 1. Ingredients and composition of ADC-05

Botanical Name	Family	Indian Name	Parts Used	Quantity %
Syzygium cumini	Myrtaceae	Jamun	Seed	20
Trigonella foenum graecum	Papilionaceae	Methi	Seed	8
Azadirachta indica	Meliaceae	Neem	Leaf	8
Emblica officinalis	Euphorbiaceous	Amla	Fruit	12
Cassia auriculata	Caesalpiniaceae	Senna	Leaf	8
Gymnema sylvestre	Asclepiadaceae	Gurmar	Leaf	20
Andrographis paniculata	Acanthaceae	Kalmegh	Leaf	8
Tribulus terrestris	Zygophylaceae	Gokhru	Fruit	8
Pterocarpus marsupium	Leuminosae	Vijasar	Berk	8

The quantification was based on The Ayurvedic Pharmacopoeia of India, Ministry of Health and Family Welfare, Department of AYUSH, Govt. of India, New Delhi, 2004.

Animals

Adult male Swiss mice and Wistar male adult albino rats were used. In the present experiments, recommended guidelines for the care and use of the animals were strictly followed [18]. The permission from Institutional Animal Ethic Committee was also obtained (IAEC/AH-2/2011/ UCM-72). The room temperature was maintained at 23°±2°C and humidity between 40 and 60%. The light cycle was also maintained (12:12h). The animals were fed supplementary feed for animal and water *ad libitum*. The food was also withdrawn as per experimental protocol.

In vitro pharmacological evaluation

Total phenolics estimation

To 0.1 ml of ADC-05 (1 mg/ml) solution in methanol, 0.5 ml of Folin-Ciocalteu reagent and 2.4 ml deionized water was added, mixed and incubated in the dark for 3 minutes. Thereafter, 2 ml of 20% sodium carbonate solution was added, mixed and further incubated in the dark for 5 min at 50ºC. The absorbance was read at 650 nm. The total phenolics in the extract were expressed as µg of Gallic acid equivalent per mg (µg GAE/mg) of extract.[19]

DPPH radical scavenging activity

0.1 ml of ADC-05 test solution at different known concentrations was mixed with 3.9 ml of 0.1 mM DPPH solution and was allowed to stand in dark for 30 minutes. The absorbance values were measured at 517 nm.[20]

α-amylase enzyme inhibition determination

The total assay mixture composed of 0.4 ml of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.02 ml of 0.04 units of PPA (porcine pancreatic α-amylase) solution and 0.08 ml of ADC-05 at concentration from 10-1000 µg/ml were incubated at 37°C for 10 min.

After pre-incubation, 0.5 ml of 1% (v/v) starch solution in buffer was added to each tube and incubated at 37°C for 15 min. The reaction was terminated with 1 ml 96 mM DNSA (3, 5-dinitrosalicylic acid) reagent, placed in boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted with 2 ml of and the absorbance measured at 540 nm. The control PPA at 0.2 U/ml represented 100% enzyme activity and did not contain any plant extract. To eliminate the absorbance produced by test compound, appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included [21]. The result was expressed as % inhibition calculated using the formula and IC_{50} values of ADC-05 was determined.

α-glucosidase enzyme inhibition determination

α-glucosidase was dissolved in 0.1 M phosphate buffer, pH 6.8 and was used as enzyme source while, 0.01 M para-nitrophenyl-α-D-glucopyranoside (PNPG) was used as substrate. The different concentration of ADC-05 (10-1000 µg/ml) in 0.5 ml of phosphate buffer (pH 6.8) was mixed with 0.05 ml of 10 mM PNPG solution in phosphate buffer and then it was incubated in 25°C for 10 min. After preincubation, 0.02 ml of buffer containing 0.5 mg/ml of the enzyme was added and further incubated at 25°C for 5 min. Finally, 0.3 ml of 50 mM sodium hydroxide was added to the mixute and the absorbance was measured at 410 nm [22]. The result was expressed as % inhibition calculated using the formula and IC_{50} values of ADC-05 was determined.

In vivo pharmacological evaluation

Acute toxicity study in mice

ADC-05 was tested for acute oral toxicity study following the guidelines of OECD No. 423 [23]. ADC-05 was given to the 18 h fasted mice in doses of 0.25 g, 0.5 g 1.0 g and 2.0 g per kg, *i.e.*, in arithmetically progressive manner by oral route in a single dose and observed for 3 days. The rate of mortality up to 72 h was recorded for the selection of 50% lethal dose of test formulation.

Streptozotocin induced type 2 diabetes in rats

STZ was dissolved in ice-cold citrate buffer (0.1 M, pH 4.9) and injected intravenously through the tail vein at the dose of 65 mg/kg to all animals (except normal control). The diabetic state was confirmed 14 day after STZ injection by measuring fasting blood glucose (one-touch glucometer, Accu-chek sensor glucometer) [24]. Rats with fasting blood glucose <250 mg/dl were considered to be diabetic and were used in for further studies. The following experiments were conducted to confirm the anti-hyperglycemic action of ADC-05. On the day 14, blood glucose was estimated in 18 h fasted rats. ADC-05 (100 mg/kg and 200 mg/kg) and standard anti-hyperglycemic drug, Glibenclamide (5 mg/kg) was given orally. Control rats were received only distilled

water. Thereafter, blood glucose was measured at 0 h, 1 h and 2 h using glucometer and compared with normal control and STZ control. All the animals were continued with the same dose of test compound (ADC-05) and glibenclamide once daily for 14 days. After the experimental regimen, the animals were fasted overnight and their blood glucose was monitored 2 h after last dose given using glucometer and compared with respective control.

Results and Discussion

Results

In vitro pharmacological evaluation

The total amount of phenolic content present in ADC-05 was 103.56 µg GAE/mg. The IC_{50} of ADC-05 on DPPH radical formation was 81.20 µg/ml. ADC-05 showed α-amylase and α-glucosidase inhibition dose dependently and its IC_{50} was 61.86 µg/ml and 34.49 µg/ml respectively (Table 2).

In vivo pharmacological evaluation

ADC-05 did not showed any signs of toxicity or mortality up to 2.0 g/kg per oral dose in mice. Further, Table 3 showed ADC-05 treatments reduced the blood glucose level significantly and dose dependently, similar to glibenclamide. STZ enhanced fasting blood level nearly three times within 14 days than STZ untreated normal control. Oral administration of ADC-05 at the dose of 100 mg/kg and 200 mg/kg declined the fasting blood sugar level 15.33% and 19.4% in 1 h and 23.55% and 30.57% in 2 h respectively in STZ induced diabetic rats. Glibenclamide treatment at the dose of 5 mg/kg lowered the fasting blood glucose concentration 23.16% in 1 h and 31.55% in 2 h in STZ rats (Table 3). Table 4 represented that two weeks of administration of ADC-05 to STZ-diabetic rats at similar two doses showed a significant reduction of blood glucose level. The percentages of blood glucose fall were 21.48% and 29.19% respectively on first week and 29.36% and 34.64% respectively on second week when compared to untreated STZ diabetic rats.

Table 2. *In vitro* antioxidant and anti-diabetic actions of ADC-05

	Phenolic content µg GAE/mg	DPPH radical scavenging IC_{50} (µg/ml)	α-amylase inhibitory activity IC_{50} (µg/ml)	α-glucosidase inhibitory activity IC_{50} (µg/ml)
ADC-05	103.56±9.42	81.20±3.01	61.86±5.95	34.49±1.26

N=6 rat in each group; Values were Mean ± SEM; GAE/mg=gallic acid equivalent/mg extract; IC_{50}= 50% inhibitory concentration

Table 3. Effect of single dose of ADC-05 on blood glucose in STZ-induced diabetic rats

	Fasting blood glucose (mg/dl)				
	0 h	1 h		2 h	
	Mean ± SEM	Mean ± SEM	% change	Mean ± SEM	% change
Normal	93.5±1.25	94±1.06	0.53	92.1±1.40	-1.49
STZ	293.5±9.32^{a***}	297.6±10.05^{a***}	1.39	295±9.96^{a***}	-0.51
GBC (5 mg/kg)	293.8±9.31^{b}	225.8±11.49^{b**}	-23.16	201.1±8.34^{b***}	-31.55
ADC-05 (100 mg/kg)	289.5±6.49^{b}	245.1±5.76^{b**}	-15.33	221.3±8.20^{b***}	-23.55
ADC-05 (200 mg/kg)	295.3±9.83^{b}	238±9.69^{b**}	-19.40	205±10.12^{b***}	-30.57

N=6 rat in each group; Values were Mean ± SEM; GBC=Glibenclamide; Data were compared statistically by t test using spss software v. 17 with respective control (p^a with normal and p^b with STZ); % change was compared to 0 h; *p<0.05, **p<0.01 and ***p<0.001 was indicates level of significant; percent change between groups.

Table 4. Effect of multiple dose of ADC-05 on blood glucose in STZ-induced diabetic rats

	Fasting blood glucose (mg/dl)				
	Day 14	Day 21		Day 28	
	Mean ± SEM	Mean ± SEM	% change	Mean ± SEM	% change
Normal	93.5±1.25	91.3±1.35	-2.35	90.3±0.71	-3.42
STZ	293.5±9.32^{a***}	322.6±13.92^{a***}	9.91	326.5±9.28^{a***}	11.24
GBC (5 mg/kg)	293.8±9.31^{b}	199.6±4.32^{b***}	-32.06	164.8±6.58^{b***}	-43.90
ADC-05 (100 mg/kg)	289.5±6.49^{b}	227.3±3.87^{b***}	-21.48	204.5±3.72^{b***}	-29.36
ADC-05 (200 mg/kg)	295.3±9.83^{b}	209.1±6.65^{b***}	-29.19	183.0±5.31^{b***}	-34.64

N=6 rat in each group; Values were Mean ± SEM; GBC=Glibenclamide; Treatment= once/day/p.o. for 14 days (day 14 to day 28); Values were Mean ± SEM; Data were compared statistically by t test using spss software v. 17 with respective group of day 14 (p-value^a with normal and p-value^b with STZ); % change was compared to Day 14; P<0.05 was considered as significant.

Discussion

Diabetic hyperglycemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells. ROS increases the generation of TNF-α expression and interleukins, which has been implicated in the pathogenesis of insulin resistance [25]. Plant polyphenolics have also been reported to inhibit α-amylase and have been shown to be the principle substance for suppressing post prandial hyperglycemia. Previous reports also support the strong antioxidant abilities of most of plants of ADC-05 [26-29]. Present study demonstrated that ADC-05 has strong scavenging abilities and inhibited the free radical formations.

It has been shown that activity of human pancreatic α-amylase and α-glucosidase in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of type-2 diabetes [30-31]. Hence, retardation of starch digestion by inhibition of enzymes such as α-amylase and α-glucosidase play key role in the control of diabetes. The search for new active agents obtained by screening natural sources such as medicinal plants or their extracts can lead to potent and specific inhibitors for α-amylase and α-glucosidase. In the present study, ADC-05 containing nine indigenous anti-diabetic medicinal plants and that was screened for its α-amylase and α-glucosidase inhibitory potential. Previous reports confirmed the α-amylase and α-glucosidase inhibitory potentialities of *A. paniculata*, *T. foenum graecum*, *T. terrestris* and *P. marsupium* [32-33]. In the present study, ADC-05 showed potent α-amylase and α-glucosidase inhibition dose dependently and may be consider potential candidates as a part of dietary and supplement designs to manage early stages of hyperglycemia linked to type-2 diabetes.

Streptozotocin (STZ) is a naturally occurring nitrosoamide that has been used extensively to produce diabetes in experimental models. In the present investigation, diabetic rats showed elevation in blood glucose levels, confirming abnormalities of glucose metabolism. ADC-05 treatments reduced the blood glucose level significantly and dose dependently, similar to glibenclamide. Present evidence clearly indicated ADC-05 has anti-hyperglycemic or blood sugar lowering action, especially during diabetic conditions. ADC-05 is composed with active anti-diabetic principles from herbal origin, like, *S. cumini*, *T. foenum graecum, A. indica, E. officinalis, C. auriculata, G. sylvestre, A. paniculata, T. terrestris* and *P. marsupium* [9-17]. All these herbs were reported to have anti-diabetic properties especially on STZ induced diabetes in rats. Even most of them have also been reported for their antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective and immunomodulatory properties, which might be helpful to combat the complications developed in diabetes. Extensive studies have also been helped to find out their active anti-diabetic constituents and also their mode of actions either through stimulate the β-cells to release of insulin or through enhance the use of glucose or its metabolism in the tissues and organs, or might scavenging the free radicals to resist lipid peroxidation, and to correct the lipid and protein metabolic disorder. Hence, ADC-05 confirmed effectiveness in diabetes in animals [34].

Conclusion

Present studies also confirmed, ADC-05 is enriched in phenolics and has strong abilities to scavenging harmful radicals. It has also inhibitory actions on α-amylase and α-glucosidase and thereby it may control or regulate the glucose absorption and its metabolism during diabetes. Therefore, ADC-05 has the therapeutic potentialities and may be useful in the management of diabetes mellitus.

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