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Anti-diabetic activity of compound isolated from *Physalis angulata* fruit extracts in alloxan induced diabetic rats

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Abstract

Excessive food consumption regarding to high calorie, obesity, cardiovascular disease, stress, and lack of exercise are risk factors for diabetes mellitus (DM). One alternative therapeutic approach in DM patients is traditional use of herbal medicines such as Physalis angulata herbs. The aim of the study was to evaluate the anti-diabetic effect of isolated compound from P. angulata in alloxan induced diabetic rats. Anti-hyperglycemic effect was investigated in normal and alloxan induced diabetic rats. Glibenclamide (150 mg/kg, p. o.) were used as reference drugs for comparison. The active compound was isolated by using chromatographic techniques. The isolated compound significantly ($P<0.05$) reduced blood sugar level in alloxan induced diabetic (hyperglycaemic) rats orally at 25 mg/kg and 50 mg/kg body weight respectively. The findings of this experimental animal study indicate that the compound isolated from P. angulata fruit extract possesses antihyperglycemic properties; and thus lend pharmacological credence to the folkloric, ethnomedical uses of the plant in the treatment and as well as in the management and/or control of type 2 diabetes mellitus.

Keywords: Physalis angulata, Diabetes mellitus, Antihyperglycemic, OGTT and herbal medicines.

INTRODUCTION

Diabetes mellitus is a term employed to describe a metabolic disorder characterized by persistent hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Holt, 2004). The long-term effects of diabetes mellitus include progressive development on the specific complications of retinopathy, nephropathy, and/or neuropathy (Nathan, 1993). People with diabetes are at increased risk of cardiovascular disease (Collins et al, 2003).

The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world and there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Akhtar et al, 2007; D Krishna Gopal, 2013).

In conventional therapy, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycemic agents (sulphonylureas, biguanides etc). In traditional practice medicinal plants are used in many countries to control diabetes mellitus. Diabetes mellitus has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. A large number of plant preparations have been reported to possess antidiabetic activity over last several decades. Researchers in India have documented the use of over 150 plants in various families with hypoglycemic activity (Patel et al, 2006; E. Sucharitha and Estari, 2013.).

Physalis angulata L is an annual, herbaceous plant which belongs to Solanaceae family. It is known by different names, including camapu; cutleaf groundcherry; wild tomato, mullaca, winter cherry etc. In Southwest Nigeria, it's known as Koropo. Its biologica properties include antimycobacterial, anticancerous, antitumorous, anticoagulant, hypotensive, immunostimulant etc (Pietro et al, 2000; Januário et al., 2002; G. Krishna et al, 2014).

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The Plant prefers moist drained sandy loamy soil with full sun or partial shade; it is renowned as an effective stimulant for the immune system. The juice is used in the treatment of earache, jaundice, fever, bladder diseases etc The fruit and other aerial parts are used in the treatment of boils, sores, cuts, constipation, intestinal and digestive problems (Van Valkenburg and Bunyapraphatsara, 2002; Devdatta Gopal Lad, 2014), and used as an antimutagenic, anticoagulant, antispasmodic, antileucemis agents. *In vivo* antitumour activity was demonstrated in mice (Chiang et al., 1992; Rajendra Prasad Gujjeti and Estari Mamidala, 2014), while it is used in treatment of hepatitis, diabetes, asthma, and malaria. It also possesses anticarcinogenic properties (Wen-Tsong et al., 2006). The present study was designed to evaluate the anti-diabetic activity of isolated compound withangulatin extract of the *Physalis angulata* fruits against alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

The fully mature *P. angulata* fruits were collected in June-July 2013 from Komatipalli Village in Warangal district of Telangana State, India. The plant fruit was identified and authenticated by Prof. V.S. Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal and voucher specimen was deposited in the metabolic disorders and infectious diseases research laboratory of the Department of Zoology, Kakatiya University.

Extraction and Isolation

The dried, powdered fruits of *P. angulata* (1.0 kg) were extracted with MeOH under room temperature for 1 week. The concentrated MeOH extract (95 g) was chromatographed on a silica gel column with a CHCl3/acetone mixture of increasing polarity as eluents, and each fraction was monitored by TLC. The fractions eluted with CHCl3/acetone (5:3) were combined and evaporated to give a residue (1.8 g) that was further chromatographed on a silica gel column with *n*-hexane/EtOAc/MeOH (8:5:0.5) as eluent. The all fractions (withanolides) were collected and further purified with preparative TLC and developed with *n*-hexane/EtOAc/MeOH (4:3:0.3) to give single compound which is a pale yellow powder (2.6 mg), which was identical to withangulatin I (Wu et al, 2004).

Animals

Wistar rats (160 - 180 g) were procured from animal house of Pharmaceutical College, Kakatiya University, Warangal for experimental study. They were acclimated to animal house conditions fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animal).

Acute toxicity studies

Acute oral toxicity (Ecobichon, 1997) study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the isolated compound administered orally at the dose level of 25 and 50 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2 - 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 100, 300 and 1000 mg/kg body weight.

Anti-diabetic evaluation

Induction of diabetic mellitus:

After fasting for 18 h, 60 rats were injected by intraperitoneally with a single dose of 150 mg/kg alloxan after dissolving it in freshly prepared normal saline. After the injection, they had free access to feed and water and were given 5% glucose solution to drink overnight to counter the hypoglycemic shock. The development of diabetes was confirmed after 48 h of the alloxan injection. The rats having fasting blood glucose level more than 200 mg/dL were selected for experimentation. From, the out of 50 animals, 8 animals were died before grouping and 6 animals were omitted from the study, because mild hyperglycemia (below 150 mg/dL). From the 36 diabetic animals, they were divided into seven groups each having 6 animals.

Collection of blood samples and glucose determination

Blood samples were collected by end tail vein cutting method and blood glucose level was determined by using one touch electronic glucometer. Using glucose strips (Lifescan, Johnson and Johnson Ltd.) (Kumar et al., 2005).

Experimental protocol

The group I consist of 6 normal control animals. The remaining each group consists of 6 Alloxan induced diabetic rats. Group I–Normal control animals received physiological saline 10 ml/kg per orally for 15 days; Group II–alloxan induced diabetic animals received physiological saline 10 ml/kg, p.o. for 15 days. Group III and IV–alloxan induced diabetic animals received isolated compound at the dose of 25 and 50 mg/kg p.o. daily for 15 days. Group V–alloxan induced diabetic animals received standard drug, glibanclamide 1.25 mg/kg daily p.o. for 15 days. All the group of animals received the treatment by the above schedule for 15 days. Blood samples were collected one hour after drug administration on the day 1, 5, 10 and 15th day to determine the blood glucose level by electronic glucometer (Babu et al 2002; Nuredin et al, 2015).

Statistical analysis:

Data obtained from pharmacological experiments are expressed as mean ± SD. Differences between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's *t*–test. p value < 0.05 were considered as significant.(Dixon and Jennrich, 1990).

RESULTS

Identification of the compound:

On isolation of the extract, the fractions containing withanolides were combined. The combined fractions were further purified by repeated column chromatography and preparative TLC to afford a minor withanolide, withangulatin.

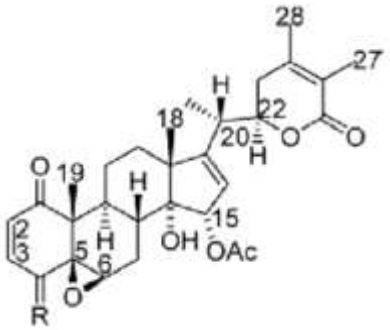

Figure-1. Structure of Withangulatin-A

The structure of this compound was established based on detailed spectral analyses, chemical transformation, and comparison with the spectral data reported in the literature. The UV spectrum showed absorption at l max (MeOH) 227 nm, which also implied the presence of alpha, beta–unsaturated ketone moieties. The molecular formula of the compound was determined to be C30H36O8 by high-resolution (HR) EI-MS at *m/z* 524.2413 and it is confirmed as Withangulatin-A.

Acute toxicity studies:

This study showed no mortality up to the dose of 2,000 mg/kg body weight. So, the extracts safe for long term administration.

Anti-diabetic activity:

The blood sugar levels measured in normal and experimental rats in initial and at the 1, 5, 10 and 15 days of treatment are given in Table 1. Alloxan-induced diabetic rats showed significant increase in the levels on blood sugar as compared to normal rats. Oral administration of the isolated compound, Withangulatin-A at a dose level of 25 and 50 mg/kg also showed significant decrease ($p<0.05$) in blood sugar level. The standard drug, glibenclamide decreased blood sugar level in 15 days treatment.

DISCUSSION

The aim of the present study was to evaluate the antidiabetic effect of isolated compound Withangulatin-A from *P. angulata*, against alloxan-induced diabetic rats. The continuous treatment of the compound of *P. angulata* for a period of 15 days produced a significant decrease in the blood sugar levels of diabetic rats. These results confirmed the use of *P. angulata* fruit of traditional practice as an anti-diabetic. The standard drug, Glibenclamide has been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic beta-cells (Tian et al., 1998). It may be suggested that the mechanism of action of Withangulatin-A is similar to glibenclamide, this is may be the first report that demonstrates antidiabetic properties for Withangulatin-A. The possible mechanism by which seed brings about a decrease in blood sugar level may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from beta cells of the islets of Langerhans or its release from the bound form. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects (Nair et al,

Table-1: Anti-diabetic activity of Physalis angulate fruit isolated compound against Alloxan-induced diabetic rats.

Group (15 days) (n=6)	Blood sugar level in mg/dL (mean ± SD)				
	Initial	Day 1	Day 5	Day 10	Day 15
Group-I	68.78±6.08	64.20±8.22	66.72±8.95	68.00±6.41	64.58±5.77
Group-II	248.78±8.54	260.24±14.54	284.5±3.78	308.20±7.09	312.10±4.67
Group-III	251.84±4.70	256.47± 5.43a^{NS}	233.54±5.60 a*b*	191.50±3.49a*b*	154.25±9.24 a*b*
Group-IV	247.38±3.20	250.17± 8.22a^{NS}	216.80±4.25 a*b*	189.10±6.79a*b*	179.21±8.58 a*b*
Group-V	249.65±8.34	246.61± 8.14a^{NS}	190.90±10.0 a*b*	167.93±8.47a*b*	122.90± 4.89a*b*

Values are mean + SD of respective groups,
*NS=Non Signficant, *p<0.05*
Comparison were made a+initial Vs day1, day 5, day 10 and day 15 of respective groups
b=Group II Vs group III to V

2006 and Vijayvargia et al, 2006; Raju Porika et al, 2014; Sateesh Poojari et al, 2014 and Vinatha Naini and Estari Mamidala, 2013). These results confirmed the use of *P. angulate* fruit in traditional system of medicine to treat diabetes in India. Further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of the hypoglycemic effect of *P. angulate* fruit.

Competing Interests Statement:

The authors declare that they have no competing financial interests.

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