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Pharmacological screening evaluation of anti diabetic activity on ethanolic extract of *Agave Angustifolia* leaves

D. Jothieswari, V. Hari, J. Kojole, V.K. Damini, S. Shaik Roshnin and Megha

Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, Andhra Pradesh – 517127, India

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ABSTRACT

The present study was designed to investigate the antidiabetic potential of the leaves of *Agave angustifolia* which has been utilized traditionally to cure diabetes mellitus. An acute toxicity study was done to check the toxicity of *Agave angustifolia* ethanolic extract and an oral glucose tolerance test (OGTT) was carried out in a study population of normoglycemic rats. The biochemical parameters considered were plasma glucose level, degree of glycosylation of hemoglobin, and peripheral consumption of glucose levels. The ethanolic extract had shown significant defence and lowered the blood glucose levels. In streptozotocin-induced diabetic rats, the highest reduction in blood glucose was observed after 2hrs at a dose level of 200 and 400 mg/kg of body weight. The streptozotocin-induced diabetic rats showed significant reductions in biochemical parameters after treatment with the extract and Glibenclamide (used as standard) as compared to the diabetic controls. The ethanolic extract of *Agave angustifolia* exhibited antidiabetic activity and its sensitivity in experimentally induced diabetic rats in a dose-dependent manner. The current results indicated the beneficial effects of the ethanolic extract of *Agave angustifolia* in both controlling hyperglycemia and the protection of the pancreatic islet cells against oxidative stress in diabetic animals.

Keywords: *Agave angustifolia,* Glibenclamide, Streptozotocin, OGTT

INTRODUCTION

Diabetes was first documented as a disease about 3000 years ago in the earliest Egypt [1]. Diabetes is quickly emerging as serious and main public health-care problem throughout the world. It is a complex and chronic illness. The terms "Diabetes" and "Mellitus" are derived from Greek [2]. Diabetes mellitus is a metabolic disturbance of carbohydrates, proteins, and fat due to relative lack or complete absence of insulin and insulin resistance [3]. It is characterized by high level of blood glucose. Diabetes mellitus was first depicted in Quite a while in the old writings of Charaka and

Address for Correspondence: Dr. D. Jothieswari, Professor & Vice Principal, Dept. of Pharmaceutical Analysis, Sri Venkateswara College of Pharmacy, RVS Nagar, Chittoor, Andhra Pradesh – 517127, India. E-mail: jothies82@gmail.com

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Sushruta[4]. In Ayurveda diabetes mellitus (DM) is alluded to as Madhumeha or Kshaudrameha, which actually implies unnecessary pee with sweet pose a flavor like nectar [5]. In the long duration, this disease can affect most of the organ systems in the body [6]. The patients of diabetes belong to all age groups. It is now considered as a major global health problem. Globally an estimated number of about 387 million people are currently diagnosed to have diabetes [7-8].

Though there are a variety of modern drugs available for getting better the hyperglycemic state, such as sulfonylureas, biguanide, thiazolidinedione, α-glucosidase inhibitors, and glinides, patients continue to suffer from the lots of side effects of these modern drugs due to which the search for new pharmacological approaches is ongoing and concerted efforts are being made to develop suitable alternative effective remedies for diabetes [9-10]. Presently, there is a growing interest in herbal remedies that are apparently efficient, produce minimal side effects, and are rich sources of antidiabetic, antihyperlipidemic, and antioxidant agents such as flavonoids, gallotannins, amino acids, and other related polyphenols [11].

Agave angustifolia belonging to the family Asparagaceae, is a perennial shrubby plant having very large rosettes of leaves. Naturalized in the coastal districts of south-eastern Queensland, though its actual distribution may be underestimated by herbarium records. Also naturalized in the coastal districts of central and northern Queensland, over and above in north-eastern New South Wales [12] *Agave angustifolia* (Caribbean agave) is native to Mexico and Central America. It grows in brushy rocky slopes, moist quebradas, or moist thickets. It is additionally planted for decoration. *Agave angustifolia* is utilized customarily in Mexico for treatment of aggravation. It is additionally utilized underway of mixed drinks, for example, mescal [13]. *Agave angustifolia* is under examination to screen for potential antioxidant activity, anti cancer activity antimicrobial activity, anti-inflammatory and in treatment of obesity and ulcers.

To our knowledge, there is no scientific evidence in the support of antidiabetic activity of *Agave angustifolia*. Hence, the present study was aimed to ascertain the scientific basis for the use of *Agave angustifolia* in the management of diabetes using streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Plant Collection and Authentication: Develop *Agave angustifolia* plants were gotten from R.V.S Nagar, Chittoor area in Andhra Pradesh province of India. The plants were distinguished and confirmed by Dr. K. Madhavachetty, Plant Taxonomist (IAAT: 357), Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Voucher Number: 1005 is kept in herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Chemicals: STZ was purchased from Loba chemicals. Glibenclamide was obtained from Sun Pharmaceutical Industries Limited. All other commercial reagents used throughout the experiments were of analytical grade.

Preparation of plant extract: Agave leaves were cut, stripped and cut into little pieces which were then dried under the sun for seven days. The dried leaves were ground into powder and sieved. The obtained powder was stored in a tightly closed amber coloured bottle Six glass bottles were taken and 200ml each of hexane, ethyl acetate, chloroform, acetone, ethanol and distilled water was added in each bottle respectively. To each bottle, 100g of the plant powder was added and soaked for 24 hrs. The mixtures were then filtered and the filtrates were subjected to evaporation using a water bath to remove the solvents. Percentage yields were calculated for each solvent and the results are as shown on Table 1. The dried extracts were used for phyto chemical screening.

Phytochemical analysis: Synthetic tests for the screening and ID of bioactive substance constituents in the restorative plants under study (*Agave angustifolia*) were completed utilizing separates arranged in various solvents by standard strategies and the outcomes are appeared in Table 2.

Acute Toxicity Study: Acute toxicity refers to adverse effects which occur within a short time after administration of a single dose or multiple doses of a substance.

Procedure: The intense poisonousness investigation of *Agave angustifolia* extricate in pale skinned person rodents was preceded according to Organization for Economic Cooperation and Development (OECD) rules (No 423). Both male and female pale skinned person rodents weighing 130-170g were utilized. Rodents were partitioned into the gatherings of 3 creatures for each gathering. A solitary portion study was directed to decide the intense poisonous of *Agave angustifolia* extricate according to OECD 423 guidelines. Rodents were fasted for the time being preceding dosing with free access to water. After the fasting meeting, the rodents were gauged and the concentrate of *Agave angustifolia* was managed to

3 rodents at a portion of 0.5 mg/kg and the creatures were watched for mortality. In this study; no mortality was observed and higher doses of 50, 300, 2000 mg/kg were employed for further toxicity studies.

The rats were then observed for clinical signs, gross behavioural changes, morbidity and mortality. Animals were also observed for occurrence of clonic convulsion, tonic extension, muscle spasm and catatonia for 1 hour, 2 hours, 4 hours,8 hours and 24 hours after administration of extracts. After observing mortalities and behavioural profile, the maximal safe dose for the study was noted. In accordance with the OECD guidelines, the doses for the study were narrowed down [6]. After effects of intense harmfulness investigation of *Agave angustifolia* were appeared in Table 3.

***Invivo* antidiabetic activity of *Agave angustifolia* leaves extract in streptozotocin induced diabetic wistar albino rats:** Wistar albino rats (150- 200 grams) of both sexes were procured .Prior to the experiment the rats were housed in a clean polypropylene cages (6 rats/ cages) for a period of 7 days under standard temperature (25 - 30o c), relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organisational Animal Ethics Committee (OAEC). The creatures were placed in for the time being fasting was denied of nourishment for 16 hrs however permitted free access of water.

Hypoglycemic Test: Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2mg /kg), Group III served as aqueous ethanolic extract of *Agave angustifolia* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Agave angustifolia*– (400mg/kg). Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test: Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat), Group II served as Positive control – Glibenclamide (2 mg /kg), Group III served as aqueous ethanolic extract of *Agave angustifolia* – (200mg/kg), Group IV served as aqueous ethanolic extract of *Agave angustifolia* – (400mg/kg). All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been adminisratered to different groups and 30mins later all the groups were glucose treated to rats orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals: A solitary portion (100 mg/kg b.w., i.p.) of streptozotocin monohydrate disintegrated in sodium citrate cushion was utilized for the enlistment of diabetes in rodents after overnight fasting. After 1 hr of streptozotocin monohydrate organization, the creatures were given feed Ad libitum and 5% dextrose arrangement was additionally given in taking care of jug for a day to beat early hypoglycaemic stage. The creatures were balanced out for a week and creatures indicating blood glucose level in excess of 200 mg/dl were chosen for the examination.

Experimental design: Five groups of rats six in each groups received the following treatment schedule for 14 days.
GROUP I - Normal control (normal saline 10 ml /kg, P.O)
GROUP II - Streptozotocin treated control (100 mg/kg, I.P)
GROUP III - Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).
GROUP IV - Streptozotocin (100 mg/kg, i.p.) + EEAAL.(200 mg/kg, P.O)
GROUP V - Streptozotocin (100 mg/kg, i.p.)+ EEAAL. (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control rats. Group IV and Group V (which previously received streptozotocin100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Agave angustifolia* group III received standard drug Glibenclamide (2 mg/kg, P.O) for 14 consecutive days. (EEAA- Ethanolic extract of *Agave angustifolia* Leaves) [14-17].

RESULTS AND DISCUSSION

Acute toxicity: The results of table 3 show the acute toxicity study, it was found that the extract induced sedation and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation.

Anti diabetic activity: The results of table 4 show half hour after the glucose treatment, all the groups

of animal blood glucose levels were significantly increased (74.5±3.863↑, 35.2±6.6905↑, 59.5±3.764↑ &65.58±3.762↑). The blood glucose levels were significantly decreased for , aqueous ethanolic extract of *Agave angsutifolia* 200 & 400 mg/kg (59.5±3.764↓& 65.58±3.762↓) ↓), (P<0.001) **& (P<0.0001) ** when compared to control and positive control at1hour and each and every½ hour blood glucose levels(200 mg/kg : 8.8±3.26,10±0,6.66±1.164 &3±0.696,400 mg/kg:17.9±1.422,9.97±0,14.7±1.093&5±0.696)(P <0.05)*, (P<0.001)**& (P<0.0001)*** were changes in the dose dependent manner extract treated group of animals compared to control and positive control but400mg/kg produce the equipotent activity. The results of Table 5 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in streptozotocin induced hyperglycaemic rats in the single dose experiment at the tested dose level and are comparable with the standard drug Glibenclamide

Table 6 show negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level (4.13±1.207↓& 1.±0.93↑) for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased glibenclamide 2mg/kg treated group (120.2±1.414↓& 23±1↓) (P<0.05)*, (P<0.001)**& (P<0.0001)*** when compared with control group at 7th and 14th days. The ethanolic leaves extract of *Agave angustifolia* treated groups 200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** (10±0.362↓& 90±1.67↓) when compared with control group but positive control have more anti diabetic activity at 7th day. The aqueous ethanolic leaves extract of *Agave angustifolia* at the dose level 400mg/kg have equipotent activity (90±1.67↓&120.2±1.414↓when compared with positive control at 7th day. The ethanolic leaves extract of *Agave angustifolia* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action (P<0.001) **& (P<0.0001) *** when compared to control and positive control. On day 14th, ethanolic leaves extract of *Agave angustifolia* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (5.1±0.07↓ & 11±1.08↓), (P<0.001) **& (P<0.0001) *** when compared to control and positive control.

Table 1: Percentage yield and consistency of *Agave angustifolia*

Parameter	Solvents					
	Hexane	Ethyl Acetate	Chloroform	Acetone	Ethanol	Aqueous
Percentage Yield	3.16	2.40	3.2	6.6	42.2	34.13
Consistency	Sticky	Sticky	Sticky	Sticky	Sticky	Sticky

Table 2: Phytochemical studies of *Agave angustifolia* extracts

S.No	Phytochemical constituents	Hexane	Ethyl Acetate	Chloroform	Acetone	Ethanol	Distilled Water
1.	Alkaloids	++	+++	++	++	+++	+++
2.	Carbohydrate	+++	+++	+++	+++	++	++
3.	Phenolic compounds	-	-	+	+	+++	++
6.	Flavonoids	+	+	+++	+	+++	++
7.	Phytosterols	-	-	-	-	-	-
8.	Tannins	+	+	++	+	+++	+

-/+ =Absence/Presence, + Less intensity, ++Moderate intensity, +++High intensity

Table 3: Acute Toxicity Studies of Ethanol Extract of *Agave angustifolia*

S.No	Treatment	Dose(mg/kg)	Sign of Toxicity	Onset of toxicity	Weight of rats		Duration of study
					Before	After	
1.	EEAA	2000	Nil	Nil	136	137	14 days
2.	EEAA	2000	Nil	Nil	145	145	14 days
3.	EEAA	2000	Nil	Nil	165	164	14 days
4.	EEAA	2000	Nil	Nil	149	150	14 days
5.	EEAA	2000	Nil	Nil	158	158	14 days
6.	EEAA	2000	Nil	Nil	162	162	14 days

Table 4: Effect of ethanolic extract of *Agave angustifolia* leaves (200 and 400mg/ kg, PO), on oral glucose tolerance test (OGTT) in normal and streptozotocin induced diabetic rats.

Treatment	Dose mg/kg	Blood Glucose Level(mg/dl) 0 min	0.5 hr	1 hr	1.5 hr	2 hr	2.5 hr	3 hr
Control Carboxymethyl Cellulose(C mc)	0.5%	68.00±2. 429	142.5±6. 292	187.5± 9.465	172.5± 12.25	157.5± 12.38	153.5± 12.83	130.2± 13.31
Positive Control Glibenclmi de	2	69.00±0. 6325	104.2±7. 323* *	110.5± 6.980 ***	93.67± 1.308 ***	83.67± 1.308 ***	77.17± 4.070* **	74.33± 2.940 ***
Aqueous Ethanolic Extract of *Agave angustifolia*	200	68.80±2. 245	128.3±6. 009	147.3± 2.404 *	138.5± 5.667 *	128.5± 5.667 *	113.8± 6.760* *	108.8± 6.107 **

The glucose levels were analyzed by using glucometer and all values are expressed as Mean±SEM (n=6), Group 2 was compared with group 1, Groups — 3,4 were compared with group 2; *p<0.05, **p<0.01,p<0.001*** evaluated by one way, ANOVA followed by Dunnet 't' test.

Table 5: Effect of single dose treatment of ethanolic extract of *Agave angustifolia* leaves on blood glucose level in normal and Streptozotocin induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)		
		0min	0.5hr	1hr
Control (Carboxy methyl cellulose)	0.5%	68.00±2.429	68.17±2.587	71.83±2.372
Positive Control (Glibenclamide)	2	69.00±0.6325	52.83±4.037**	32.83±1.515***
Aqueous ethanolic extract of *Agave angustifolia* leaves	200	68.80±2.245	60.17±3.47*	59.33±3.584*
Aqueous ethanolic extract of *Agave angustifolia* leaves	400	68.00±2.429	53.00±2.309**	34.17±1.138***

The hypoglycemic test results have shown Table 5, which indicated aqueous ethanolic extract of *Agave angustifolia* treated animals 200 & 400, significantly decreased in blood glucose level (0.84± 1093↓ & 18.83±3.879↓) (P<0.001)**& (P<0.001)*** when compared to control and positive control

Table 6: Effect of multiple dose treatment of ethanolic extract of *Agave angustifolia* leaves (Once daily), on blood glucose level after 15 days in normal and Streptozotocin induced diabetic rats

S.No	Treatment	Blood glucose level (mg/dl); Day 1	Blood glucose level (mg/dl); Day 7	Blood glucose level (mg/dl); Day 15
1	Normal control 10 ml/kg P.O	79.83±2.833	75.7±4.014	76.7± 4.944
2	Negative control	265.2±3.85	270.1±2.9	275.2±2.5 3
3	Positive control (Glibenclamide 2mg/kg) P.O	255.83±2.386	135.63±3.8***	112±2.8***
4	EEAA200 mg/kg P.O	260±3.5	250.3±3.138**	250.3±3.138**
5	EEAA 400 mg/kg P.O	263±4.55	173.1±2.88***	162.1±1.8***

The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

CONCLUSION

The ethanolic extract of *Agave angustifolia* aerial parts exhibited significant hypoglycemic activity in streptozotocin induced diabetic rats. From the phytochemical analysis it was found that the major chemical constituent of the extract was Alkaloids & flavanoids. On the basis of above evidence it is possible that the presence of Alkaloids & flavanoids may be responsible for the observed antidiabetic activity. Thus, the Alkaloids & flavanoids in the extract may be suspected to possess the activity that may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defence contributing to the protection against oxidative damage in streptozotocin induced diabetes. Further pharmacological and biochemical examination are in progress to find out the active constituents responsible for antidiabetic activity and to elucidate its mechanism of action.

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