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Pharmacological Evaluation of Aqueous And Alcoholic Extracts of *Alovera* Leaves for Evaluation of Anti-Diabetic Activity

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ABSTRACT

Crude aqueous and alcoholic extracts of leaves of *Alovera* at a dose of 20 and 30mg/kg showed significant effect on the glucose tolerance of rats and it also showed reduction in the fasting blood glucose levels of the normoglycaemic rats, thus revealing the hypoglycaemic nature of the extracts. The effect was more pronounced for both extracts. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. he data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in aqueous and alcoholic extract. The administration of different extracts at a dose of 20 and 30 mg/kg showed significant anti-hyperglycaemic effect at 22nd day which was evident from the 7th day on wards as compared to standard. The aqueous and alcoholic extract of *Alovera* has showed better anti- hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats are shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 22nd Day compare to the initial. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight shows significant (P*<0.05), effect.

Keywords: Alloxan,diabetic activity, Glibenclamide

Introduction

Protein glycation is considered one of the main causes of complications of diabetes, such as vasculopathy, retinopathy, nephropathy and neuropathy, cataracts, and chronic kidney disease [1]. The glycation of proteins brings about the formation of advanced glycation end products (AGEs), which modify the structure of proteins and alter enzymatic activity [2]. AGEs are formed by the nonenzymatic reaction of the free amino group of a protein with the carbonyl of a sugar or a reducing aldehyde (called the Maillard reaction) [3], resulting in the formation of Schiff bases, subsequently through a series of reactions, and fluorescent compounds such as pentosidine and nonfluorescent compounds such as carboxymethyl lysine (CML) are formed [4]. Some AGEs, such as CML and pentosidine, have become biomarkers for glycooxidizing damage [5]. Therefore, taking into account the pathological implications of glycation, it becomes essential to discover inhibitors of protein glycation, which may help reduce and/or prevent complications in diabetes mellitus [2]. For centuries, herbal medicines have been widely used to treat a wide variety of diseases. To date, many of these plants are still used as a first alternative to cure certain diseases in developing countries around the world due to the few side effects they present; it has also been reported that around 20% of the medicines used throughout the world come from plants [6]. In this context, there is a growing interest in discovering safe and nontoxic plant sources to find alternative or complementary medicines that help the treatment of various chronic diseases such as diabetes mellitus. *Aloe vera* (L.) Burm.f. has been used by different cultures such as the Egyptian, Indian, Chinese, and European cultures for more than 5000 years due to its extraordinary medicinal properties [7, 8]. The genus *Aloe* grows in arid, tropical, and subtropical areas; this genus includes approximately 450 species. It is a succulent plant with no stem or a short stem and can grow to be 60−100 cm high; its leaves are fleshy, thick, triangular, and spiny [8], which gives the appearance of cactus, but in fact it belongs to the lilac (Liliacea) family. Its leaves have the ability to retain water, which allows the plant to survive in environments with long periods of drought, where most of the vegetation disappears [9]. It contains more than 70 active compounds [7], including vitamins, minerals, enzymes, polysaccharides, phenolic compounds, and organic acids. It has been reported that the polysaccharides present in the *A. vera* gel have therapeutic properties such as anti-inflammatory, healing, antibacterial, antioxidant, anticarcinogenic, antidiabetic, and antiaging properties, among others [8, 9].

METHODOLOGY

Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Experimental animals

Healthy adult albino wistar rats weighing 200-250grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house. Animal studies had approval of IAEC.

Plant Material Collection and Preparation of plant extracts(Rojini Bista, et al., 2020)

The aerial parts of *Aloe barbadensis* was collected fromGarden at home. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India.

Preliminary Qualitative Phytochemical Screening of Plant Specimen

Preliminary phytochemical analysis of the extracts
The extracts so obtained were subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytoconstituents as follows:

Preparation of Aqueous Extract

The aqueous extraction is done by taking 50 grams of the plant leaf geland mixed with 200 ml of distilled water in a beaker. The mixture is heated on a hot plate at 30℃-40℃ and mixed with continuous stirring for 20 minutes. The mixture is filtered using Whatmann filter paper and the filtrate is used for further preliminary phytochemical analysis.

Qualitative Analysis for Phytochemicals

The plant methanolic extracts were screened for the presence of the phytochemical classes by using the standard methods [14].

4.6 Pharmacological evaluation

Preparation of extracts:

All the drugs were administered orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

ACUTE ORAL TOXICITY:

The acute oral toxicity of aqueous and alcoholic extracts of *Alovera* was determined by using Albino wistar rats (200-250g) which were maintained under standard conditions. The animals were fasted 12 hour prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.

Assessment of Anti-diabetic Activity in Normal and Alloxan induced Rats

Assessment of hypoglycemic activity on normal rats. Table 1----.Group Classification:

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10mg/kg
Group 2	Standard group received Glibenclamide	10mg/kg
Group 3	Aqueous extract of *Alovera*	20 mg/kg
Group 4	Aqueous extract of *Alovera*	30 mg/kg
Group 5	Alcoholic extract of *Alovera*	20 mg/kg
Group 6	Alcoholic extract of *Alovera*	30 mg/kg

Procedure:

Animals were divided randomly into six groups of four and each was fasted to overnight. The blood samples were withdrawn by tail vein at 0hour i.e. before I.P administration of extracts/standard/vehicle. Then blood was collected at an interval of 1, 2, 4, and 8 hour after the administration on 0^{th}, 7^{th}, 14^{th} and 21^{st} day respectively according to procedure blood glucose levels were measured by glucometer (ONE TOUCH glucometer).

Oral glucose tolerance test(OGTT) in normal rats:

On the next day (1^{st}, 8^{th}, 15^{th} and 22^{nd} day) after the assessment of hypoglycemic activity OGTT was carried out in same normal animals.

Procedure:

All the animals in each group were administered 2g/kg of glucose one hour after extract/ glibenclamide/ vehicle administration. The blood samples were collected by tail vein at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of glucose load. Blood glucose levels were measured by glucometer on 1^{st}, 8^{th}, 15th and 22^{nd} day respectively.

Assessment of Anti-Diabetic Activity in Alloxan Induced Diabetic Rats: Induction of Diabetes:

Albino wistar rats of either sex weighing 200-250 g were selected for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages.

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 13 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

Table 2 -.Group Classification:

Group	**Treatment**	**Dose(mg/kg)**
Group 1	Normal control received distilled water	10mg/kg
Group 2	Diabetic control received distilled water	10mg/kg
Group 3	Standard group received Glibenclamide	10mg/kg
Group 4	Aqueous extract of *Alovera*	20mg/kg
Group 5	Aqueous extract of *Alovera*	30mg/kg
Group 6	Alcoholic extract of *Alovera*	20 mg/kg
Group 7	Alcoholic extract of *Alovera*	30 mg/kg

Effect of Aqueous and Alcoholic extracts of *Alovera* on blood glucose levels in alloxan induced diabetic rats:

All the animals of above groups were administered as per treatment protocol mentioned above. The blood samples were collected by retro orbital puncture at 0,1,2,4 and 8 hour after the administration. The treatment was continued for next 22 days. Again blood samples were also collected on 7^{th}, 14^{th} and 21^{st} day after 1 hour administration for sub acute study. Blood glucose level was measured by glucometer at various time intervals.

Oral glucose tolerance test (OGTT) in alloxan induced diabetic rats:

On the 8^{th}, 15^{th} and 22^{nd} day OGTT was carried out on the same alloxan induced diabetic animals used for assessment of anti-diabetic activity studies.

Procedure:

All the animals in each group were administered 2g/kg of glucose one hour after extract/ Glibenclamide/ vehicle administration. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of the glucose load. The Blood samples were collected by tail vein and its blood glucose levels were measured by using a glucometer apparatus.

Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparison had made. i.e.

1. Normal control Vs All treated groups.
2. Diabetic Control Vs All treated groups.

Differences between groups were considered significant at $P<0.001$ and $P<0.05$ levels.

Phytochemical screening of *Alovera*.

The aqueous extract of *Aloe vera* was prepared to conduct preliminary phytochemical analysis.
The phytochemicals screening of aqueous extract of Aloe Vera showed that bioactive compounds such as flavonoids, steroids, terpenoids, proteins, phenols, carbohydrates, reducing sugar, starch, tannins, glycosides were detected to be present in the leaves of Aloe Vera whereas saponin was negative as shown in Table 1. These findings are in total agreement with those existing in the literature Other studies have shown that the presence of saponins depends on extraction solvents. They are positive with ethanol, methanol, ethyl acetate, petroleum ether, acetone and hexane extracts and negative with the aqueous extract.

Acute toxicity testing

Acute toxicity studies revealed that the alcoholic extracts of*Alovera*were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

HYPOGLYCEMIC ACTIVITY IN NORMAL RATS

Fasting Blood Glucose Levels (FBGL) were within the range of 90-105 mg/dl in all the groups at 0day. Repeated treatment with the doses of aqueous and alcoholic extract (100 and 200 mg/kg) significantly decrease the blood glucose level on 7^{th}, 14^{th} and 21^{st} day, indicating that the extract produce significant hypoglycemic activity after repeated administration. Glibenclamide (10mg/kg) also significantly reduced Fasting Blood Glucose Level (FBGL) after repeated administration as compare to normal control group. Changes in FBGL in different groups after repeated dose administration are summarized in Table No: 3

Repeated administration of both aqueous and alcoholic extracts had significantly ($p<0.005$) reduced the FBGL on 7^{th}, 15^{th} and 21^{st} day, indicating these extracts can produce hypoglycemia on repeated administration. However hypoglycemic activity was more significant on 7^{th}, 14^{th} and 21^{st} day for Glibenclamide treated as compare with other groups. The results suggest that the both aqueous and alcoholic extracts possess significant hypoglycemic activity after repeated dose administration. The detailed results are summarized in TableNo: 3

Effect of extracts of *Alovera*on fasting blood glucose level (FBGL) in normal rats

Table No: 3- Effect of extracts of *Alovera*on fasting blood glucose level (FBGL) in normal rats.

Treatment	**Dose (mg/kg)**	**Blood glucose level(mg/dl)**		
		7^{th} day	**14^{th} day**	**21^{st} day**
Normal control	-	87.18±2.63	76.59±1.89	71.18±2.63
Glibenclamide	10	82.75±4.28	74.35±1.96	70.12±1.53
AQAV1	20	89.17±1.60	82.58±2.15	78.86±5.46
AQAV2	30	83.52±4.98	76.17±2.95	71.15±3.75
ALAV1	20	76.7±2.91	65.85±1.38	60.17±6.78
ALAV2	30	86.2±9.70	76.14±6.18	70.18±5.35

Values are expressed as mean± S.E.M. n=6. Significant values were compared with $p<0.005$, normal control Vs all groups. Parent thesis indicates % reduction in BGL.

Oral glucose tolerance test (OGTT) -

Both the aqueous and alcoholic extracts of *Aloveras*ignificantly (P<0.005) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and up to 2hr time period as compare with other groups extract Glibenclamide on 8^{th}, 15^{th} and 22^{nd} day. While aqueous and alcoholic extracts produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.005) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in Table No: 4

Table No: 4- Effect of extracts of *Aloveraon* 8^{th}, 15^{th} and 22^{nd} day in normal rats.

Treatment	**Dose (mg/kg)**	**Blood glucose level(mg/dl)**		
		8^{th} day	**15^{th} day**	**22^{st} day**
Normal control	-	89.12±5.15	82.75±1.14	75.36±2.79
Glibenclamide	10	85.17±3.58	75.14±2.93	70.96±4.35
AQAV1	20	86.35±5.18	78.15±1.72	68.52±4.14
AQAV2	30	83.12±3.79	77.37±2.83	65.12±3.65
ALAV1	20	90.35±2.56	82.12±3.96	72.43±2.34
ALAV2	30	75.86±2.42	65.28±2.75	58.99±2.10

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

ANTI-DIABETIC ACTIVITY IN ALLOXAN INDUCED DIABETIC RATS

Fasting blood glucose levels (FBGL) in normal rats were in range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to range of 252- 266 mg/dl after 72 hours. These values on subsequent days got stabilized by day seven on an average between 255 mg/dl.
Changes in the fasting blood glucose levels in different groups are tabulated in Table No: 11. This data shown that blood glucose level of normal control animals has maintained throughout the study period.
The diabetic control group has shown significant increase in fasting blood glucose levels during this 21^{st} day study period. Glibenclamide (10mg/kg) treated group has shown ($p<0.05$) significant decrease in fasting blood glucose level during 7^{th}, 14^{th} and 21^{st} day of study period.

Effect of *Alovera*extracts on antidiabetic activity in alloxan induced diabetic rats

The animals treated with 100 and 200mg/kg of aqueous and alcoholic of different extracts shown significant decrease ($P<0.05$) in FBGL on 7^{th}, 14^{th} and 21^{st} day of treatment when compare to other groups of animals. The aqueous extracts have reduced more(%) in FBGL when compared to alcoholic extracts except standard group. The detailed results are summarized in TableNo: 5

Table No: 5- Effect of extracts of *Alovera*on fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.

Treatment	**Dose (mg/kg)**	**Blood glucose level(mg/dl)**		
		7^{th} day	**14^{th} day**	**21^{st} day**
Normal control	-	85.70±6.90	75.82±5.15	68.36±2.98
Diabetic control	**10**	296.15±21.53	272.52±12.15	265.58±24.39
Glibenclamide	**10**	263.10±25.19	246.14±85.28	228.14±52.76
AQAV1	**20**	375.29±68.17	360.35±15.85	332.74±12.63
AQAV2	**30**	381.26±15.89	368.24±19.85	351.23±21.96
ALAV1	**20**	292.15±86.75	271.15±59.13	221.85±36.15
ALAV2	**30**	223.52±16.85	185.15±02.61	153.75±55.89

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with $P<0.05$. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

Oral glucose tolerance test (OGTT) on 8^{th}, 15^{th} and 22^{nd} day-

Both the aqueous and alcoholic extracts of *Alovera*are significantly ($P<0.05$) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and upto 2hr time period as compare with other groups extract Glibenclamideon 8^{th},15^{th} and 22^{nd} day. While aqueous and alcoholic extracts

produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.05) significant suppression in FBGL rise at first half-an-hour, 1hr and normalized FBGL within 2hr. The detailed results are summarized in TableNo: 6.

Table No: 6- Effect of extracts of *Aloverao*n 8th,15th and 22nd day in Diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		8th day	15th day	22st day
Normal control	-	85.12±2.85	75.28±1.91	63.14±2.89
Diabetic control	**10**	283.25±11.71	251.14±20.95	221.39±19.86
Glibenclamide	**10**	365.89±75.50	286.15±39.52	275.93±15.78
AQAV1	**20**	262.13±72.89	232.71±25.53	198.17±13.99
AQAV2	**30**	283.82±10.27	262.13±10.78	242.89±15.32
ALAV1	**20**	264.18±93.56	221.80±96.15	186.55±11.89
ALAV2	**30**	363.12±10.28	321.18±25.98	282.15±19.12

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.05. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

DISCUSSION

Despite the fact that diabetes has high prevalence, morbidity and mortality globally, it is regarded as non curable but controllable disease. Different synthetic drugs, plant remedies and dietary modification play an effective role in the reduction of the suffering that it causes. The potential role of medicinal plants as antidiabetic agents has been reviewed by several authors. In order to identify the plants with antidiabetic properties various plants have been tested *in-vivo* using animal models, for example rats,against the complications caused by inducers of diabetes, and it has been established that many plants possesses the potential to lower the fasting blood glucose levels and besides help in improving other diabetic complications. The sustained reduction in hyperglycemia automatically decreases the risk of other major complications of diabetes. Effective glucose control is the key for preventing or reversing the diabetic complications and improving the quality of life of the diabetics.

Many natural active compounds have been isolated from plants of different species. These active principles are complex Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins,Triterpenes, Sterols, Coumarin and others. These compounds have been shown to produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities. These effects might be achieved by facilitating insulin release from pancreatic ß-cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/ or increasing glucose utilization by the body. These compounds may also exhibit Anti-

Inflammatory, Antibacterial,Antifungal and Cardioprotective activities, and restore enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage.

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β - cells of islets of langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 22 days treatment. The difference observed between the initial and final fasting serum glucose levels of extract treated hyperglycemic rat's revealed antihyperglycemic effect of leaves of *Alovera* throughout the period of study. The effect of the extracts was compared to that of reference standard, Glibenclamide and was found to be significant.

Phytochemical analysis of extracts of leaves of *Alovera* revealed the presence of secondary metabolites that have been shown to possess antidiabetic effect in other plants. Flavonoids, alkaloidsandSteroids which were responsible for the antidiabetic effect in other plants were also detected in the extracts of this plant. The presence of phenols in the plant could also be responsible for the antidiabetic effect have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes. Extracts of leaves of *Alovera* appear to be attractive materials for further studies leading to possible drug development for diabetes. Development of phytomedicines is relatively inexpensive and less time consuming; it is more suited to our economic conditions than allopathic drug development which is more expensive and spread over several years.

CONCLUSION

The study was performed to find out the beneficial effects of two different extracts of leaves of Alovera in normoglycaemic rats and alloxan induced diabetic rats and the results reveal that the plant has beneficial effects on blood glucose levels.

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of leaves of Alovera. Alkaloids, Tannins, Anthraquinones, Flavonoids, Saponins, Triterpenes, Sterols, Coumarin.

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in aqueous and alcoholic extract. The administration of different extracts at a dose of 20 and 30 mg/kg showed significant anti-hyperglycaemic effect at 22^{nd} day which was evident from the 7^{th} day on wards as compared to standard. The aqueous and alcoholic extract of *Alovera* has showed better anti- hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats are shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 22^{nd} Day compare to the initial. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight shows significant ($P^{*}<0.05$), effect.

Results of anti-diabetic activity in normal and alloxan induced rats the extracts established the scientific basis for the utility of these plants in the treatment of diabetes. The extracts have shown significant reduction in blood glucose levels in normal and alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than the hypoglycaemic activity of Glibenclamide in the diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. In conclusion, these extract showed significant anti-diabetic effect in normal and diabetic rats after administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of these plants in the treatment of diabetes stands confirms.

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