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RESEARCH ARTICLE

# Pharmacological Screening and Phytochemical Evaluation of Antidiabetic Activity of *Asparagasus Racemosus* Leaves in Normal and Alloxan-Induced Diabetic Rats

Shaik. Farhatunnisa Begum, Atluri Bhavana, K. Padmalatha

*Department of Pharmacology, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Andhra Pradesh, India*

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## ABSTRACT

Diabetes mellitus is the most common endocrine disorder, affecting more than 300 million people worldwide. These therapies developed along the principles of allopathic are often limited in efficacy, carry the risk of adverse effects, and are often too costly, especially for the developing world. To identify complementary or alternative approaches to existing medications, we studied the antidiabetic potential of leaves of *Asparagus racemosus*. The acute oral toxicity studies of the extracts revealed no toxic effects up to the levels of 2000 mg/kg body weight. The aqueous and alcoholic extracts of 20 and 30 mg/kg body weight of *A. racemosus* were screened for the presence of hypoglycemic and antidiabetic activity. In this study, diabetes was induced by a single intraperitoneal dose alloxan monohydrate in 72 h fasted rats. The fasting blood glucose level (FBGL) was carried on the 7$^{th}$, 14$^{th}$, and 21$^{st}$, day and oral glucose tolerance test (OGTT) was measured on the 8$^{th}$, 15$^{th}$, and 22$^{nd}$ day. Glibenclamide was taken as the standard and the results are quite comparable with it. The studies were indicated that the leaves of *A. racemosus* are effective in the regeneration of insulin-secreting β-cells and thus possess antidiabetic activity. The aqueous and alcoholic extracts showed a significant effect in decreasing the FBGL and OGTT of rats and it's also showed good hypoglycemic activity in normal glycemic rats. The preliminary phytochemical analysis of the extracts of *A. racemosus* revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics, and glycosides as the possible biologically active principles.

**Keywords:** *Asparagus racemosus*, alloxan monohydrate, glibenclamide, fasting blood glucose level and oral glucose tolerance test

## INTRODUCTION

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be. Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and all age groups, the general public has a concern about its control and treatment.[1]

***Corresponding Author:**
Shaik. Farhatunnisa Begum,
E-mail: atluri.bhavana@gmail.com

The term "diabetes" was first used around 250 B.C. It is a Greek word meaning "to syphon," reflecting how diabetes seemed to rapidly drain fluid from the affected individual. The Greek physician Aretaeus noted that affected individuals passed increasing amounts of urine as if there was "liquefaction of flesh and bones into urine." The complete term "diabetes mellitus" was coined in 1674 by Thomas Willis. Mellitus is Latin for honey, which is how Willis described the urine of diabetics.[2]

Historical accounts reveal that as early as 700–200 BC, diabetes mellitus was a well-recognized disease in India and was even distinguished into two types, a genetically based disorder and another one resulting from dietary indiscretion. Ancient Hindu writings document how black ants and flies were attracted to the urine of diabetics. The Indian physician Sushruta in 400 B.C. described the sweet taste of urine from affected individuals, and for many centuries to come, the sweet taste of urine was a key to the diagnosis.[3]

Physicians have observed the effects of diabetes for thousands of years. One of the effects of diabetes is the presence of glucose in the urine (glucosuria). For much of the time, little was known about this fatal disease that caused weight loss of body, extreme thirst, and frequent urination. It was in 1922 that the first patient was successfully treated with insulin. Till the mid-1800s, the treatments offered for diabetes varied tremendously. A breakthrough in the puzzle of diabetes came in 1889. German physicians Joseph von Mering and Oskar Minkowski surgically removed the pancreas from dogs. The dogs immediately developed diabetes. Now that a link was established between the pancreas and diabetes, research focused on isolating the pancreatic extract that could treat diabetes. Dr. Frederick Banting succeeded in his experiments of isolating a pancreatic extract. The diabetic dog was kept alive for 8 days by regular injections until supplies of the extract, at that time called "isletin," were exhausted. Experiments on dogs showed that extracts from the pancreas caused a drop in blood sugar, caused glucose in the urine to disappear, and produced a marked improvement in clinical condition.

A young boy, Leonard Thompson, was the first patient to receive insulin treatment in the year 1922 and lived for 13 years. Over the next 70 years, insulin was further refined and purified. A revolution came with the production of recombinant human DNA insulin in 1978. Instead of collecting insulin from animals, new human insulin could be synthesized. In 1923, Banting and Macleod were awarded the Nobel Prize for the discovery of insulin. In his Nobel Lecture, Banting concluded the following about their discovery: "Insulin is not a cure for diabetes; it is a treatment."

Present status projects that the incidence of diabetes is on the rise. The present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization; there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in the year 2001, about 441,004 deaths were registered and 49,855 of them were provoked by diabetes, representing 11.2% of the total population. In the United States, diabetes is the sixth leading cause of death. The prevalence of diabetes mellitus is rapidly increasing worldwide and India is estimated to have 31 million diabetics from the total population of the world. Diabetes is predicted to become one of the most common diseases in the world within a couple of decades, affecting at least half a billion people.[4]

The driving force behind the high prevalence of diabetes is the rise of obesity, sedentary lifestyle, and consumption of energy-rich diet. The diabetes epidemic is accelerating in the developing world, with an increasing proportion of affected people in younger age groups.[5]

The prevalence of Type 2 diabetes is now at epidemic proportions. Type 2 diabetes has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. Type 2 diabetes accounts for about 90-95 % of the population while Type 1 diabetes accounts for about 5–10% of the total population. In the past, Type 2 was rarely seen in the young, but recent reports describe Type 2 diabetes being diagnosed even in children and adolescent.[6]

## Plant Profile

| | | |
|---|---|---|
| Botanical Name | : | *Asparagus racemosus* |
| Kingdom | : | Planate |
| Order | : | Asparagales |
| Family | : | Asparagaceae |
| Genus | : | Asparagus |
| Species | : | *Asparagus racemosus* |
| Common Name | : | Shatavari, Shatuli, Vrishya, Kurilo. |

It is a species of asparagus common throughout Nepal, Sri Lanka, India, and the Himalayas. It grows one to two meters tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1300–1400 m elevation). Asparagamine A (a polycyclic alkaloid), Two new steroidal saponins, shatavaroside A and shatavaroside B together with a known saponin, filiasparoside C, were isolated from the roots of *Asparagus racemosus*.[7] Five steroidal saponins, shatavarins VI-X, together with five known saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, and immunoside and schidigerasaponin D5 (or asparanin A), have been isolated from the roots of *A. racemosus* also known as the isoflavone 8-methoxy-5, 6, 4'-trihydroxyisoflavone 7-O-beta-D-glucopyranoside. *A. racemosus* (Shatavari) is recommended in Ayurvedic texts for the prevention and treatment of gastric ulcers, dyspepsia, and as a galactagogue. *A. racemosus* has also been used by some Ayurvedic practitioners for nervous disorders.[8]

## MATERIALS AND METHODS

The designing of the methodology involves a series of steps taken systematically to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from a field trip to the observation including selection and collection of the medicinal plant, selection of dose value, standardization of protocol, usage of instruments, preparation of reagents, selection of specific solvents for extraction, formation of protocols, and final execution of the standardized protocol. All this requires a good build of mind and a good and soft technical hand to handle the materials and procedure in a truly scientific manner.

### Drugs and Chemicals

Drugs and Chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India are mentioned in Tables 1-3.

### Experimental animals

Healthy adult Albino Wister rats weighing 200–250 g of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with a standard pellet diet (Amrut Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of the experiment, after 72 h of fasting from the day of alloxan introduction. Animals were housed within the departmental animal house, and the room temperature was maintained at 27°C. Animal studies had the approval of the Institutional Animal Ethic Committee with the approval number VIPW/IAEC/1581/PO/Re/11/CPCSEA/M.Ph/004/2020-21.

### Plant Material Collection

The leaves of *A. racemosus* were collected from the local market in Hyderabad in January and were identified and authenticated by the Department of Pharmacognosy. The plant material was cleaned, reduced to small fragments, air-dried under shade at room temperature, and coarsely powdered in a mixer. The powdered material was stored or taken up for the extraction process.

### Preparation of Plant Extracts

#### *Preparation of aqueous extract*

Dried leaves of *A. racemosus* were taken about 20 g into a 250 ml beaker containing 200 ml of water. The contents were mixed well and then the mixture was boiled up to 80–90°C for 4–5 h. Further, the extract was filtered with Whatman filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for the further experiment to check the activities.

***Preparation of alcoholic extract***

Dried leaves of *A. racemosus* were taken about 20 g into a 250 ml beaker containing 200 ml of Alcohol. The contents were mixed well and then the mixture was boiled up to 50–60°C for 4–5 h. Further, the extract was filtered with Whatman filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for the further experiment to check the activities.[8]

## 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:

***Alkaloids***

Extracts were dissolved individually in dilute hydrochloric acid and filtered.

a. **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). The formation of a yellow-colored precipitate indicates the presence of alkaloids.
b. **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitate indicates the presence of alkaloids.
c. **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). The formation of a red precipitate indicates the presence of alkaloids.
d. **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). The presence of alkaloids was confirmed by the formation of a yellow-colored precipitate.

***Triterpenoids***

a. **Salkowski's Test:** The extracts were treated with chloroform and filtered separately. The filtrate was treated with a few drops of concentrated sulfuric acid, shaken, and allowed to stand. If the lower layer turns red, sterols are present. If the lower layer turns, golden yellow triterpenes are present.

***Saponins***

a. **Froth Test:** The extracts were diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. The formation of a 1 cm layer of foam indicates the presence of saponins.
b. **Liebermann-Burchard Test:** The extracts were treated with chloroform and filtered. The filtrates were treated with the few drops of acetic anhydride boiled and cooled. Concentrated sulfuric acid was added through the sides of the test tube. The formation of a brown ring at the junction indicated the presence of steroidal saponins.

***Flavonoids***

**a. Alkaline reagent Test:** The extracts were treated with few drops of sodium hydroxide separately. The formation of intense yellow colorless on the addition of few drops of dilute acid indicates the presence of flavonoids.
b. **Lead acetate Test:** The extracts were treated with the few drops of lead acetate solution. The formation of a yellow precipitate indicates the presence of flavonoids.

***Phenolic and tannins***

a. **Ferric chloride Test:** The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish-black color indicates the presence of phenolics nucleus.
b. **Gelatin Test:** To the extract, 1%gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins.
c. **Vanillin hydrochloride Test:** the extracts were treated with few drops of vanillin hydrochloride reagent. The conformation of pinkish-red color indicates the presence of tannins.

## Selection of Dose For Animal Study

The dose considered for the experiment on rats was obtained from the conversion of the human dose of

*A. racemosus* (3–5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence, the calculated dose for the rats (considering human dose 0.3 and 0.5 g/kg) is 20 and 30 mg/kg. Acute toxicity was done at a dose of 2000 mg/kg body weight.[9]

## Pharmacological Evaluation

### *Preparation of extracts*

The aqueous and alcoholic extracts of *A. racemosus* were suspended in water in the presence of 3%v/v Tween-80 solution. All the drugs were administered orally for experimental purposes. Each time preparation of the extracts were prepared when required. The drugs were administered at a constant volume of 10 ml/kg for each animal.[10]

### *Acute oral toxicity*

The acute oral toxicity of aqueous and alcoholic extracts of *A. racemosus* was determined by using Albino Wister rats (200–250 g) which were maintained under standard conditions. The animals were fasted 12 h before the experiment, up-and-down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with a single dose of individual extract up to 2000mg/kg and observed for its mortality during 2 days and 7 days study period (short term) toxicity and observed up to 7 days for their mortality, behavioral, and neurological profiles.[11]

## Assessment of Antiaiabetic Activity in Normal and Alloxan-Induced Rats

### *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 0 h i.e. before I.P administration of extracts/standard/vehicle. Then blood was collected at an interval of 1, 2, 4, and 8 h 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 the same normal animals.

### *Procedure*

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

## Assessment of Antiaiabetic Activity in Alloxan-Induced Diabetic Rats

### *Induction of diabetes*

Albino Wister 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 16 h fasted rats by a single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

The rats after alloxanization were given 5% w/v glucose solution in feeding bottles for the next 24 h in their cages to prevent hypoglycemia. After 72 h, rats with fasting blood glucose levels (FBGL) >200 mg/dl were selected and used for further studies

All the animals were observed for 7 days for consistent hyperglycemia (FBGL >200 mg/dl and <400 mg/dl), and such animals were selected and divided into six groups of four each and used for the study of the following experimental models.

### *Effect of Aqueous and Alcoholic Extracts of A. racemosus on Blood Glucose Levels in Alloxan-Induced Diabetic Rats*

All the animals of the above groups were administered as per the treatment protocol mentioned above. The blood samples were

collected by a retro-orbital puncture at 0, 1, 2, 4, and 8 h after the administration. The treatment was continued for the next 22 days. Again blood samples were also collected on the 7th, 14th, and 21st day after 1-h administration for subacute study. Blood glucose level was measured by a glucometer at various time intervals.

***OGTT in Alloxan-Induced Diabetic Rats***

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

***Procedure***

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

**Statistical Analysis**

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

a. Normal control versus All treated groups.
b. Diabetic Control versus All treated groups.

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

## RESULTS

**Phytochemical Screening of *A. racemosus***

The present investigation concluded that the isolated compounds from the plant *A. racemosus* show the various Pharmacological effects was determined due to the presence of different phytochemical compounds Table 4. Further, study is needed for the isolation of the constituents present in the plant and its pharmacological activity should need to consider and ultimately it should be implemented for the benefit of human beings.

**Acute Toxicity Testing**

Acute toxicity studies revealed that the alcoholic extracts of *A. racemosus* 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**

FBGL were within the range of 90–105 mg/dl in all the groups at 0 day. Repeated treatment with the doses of aqueous and alcoholic extract (100 and 200 mg/kg) significantly decrease the blood glucose level on the 7th, 14th, and 21st day, indicating that the extract produces significant hypoglycemic activity after repeated administration. Glibenclamide (10mg/kg) also significantly reduced FBGL after repeated administration as compared to the normal control group. Changes in FBGL in different groups after repeated-dose administration are summarized in Table 1.

Repeated administration of both aqueous and alcoholic extracts had significantly ($P < 0.005$) reduced the FBGL on the 7th, 15th, and 21st day, indicating these extracts can produce hypoglycemia on repeated administration. However hypoglycemic activity was more significant on the 7th, 14th, and 21st day for glibenclamide treated as compared with other groups. The results suggest that both aqueous and alcoholic extracts possess significant hypoglycemic activity after repeated-dose administration. The detailed results are summarized in Table 6.

**Effect of Extracts of *A. Racemosus* on FBGL in Normal Rats**

**OGTT**

Both the aqueous and alcoholic extracts of *A. racemosus* significantly ($P < 0.005$) suppress the rise in FBGL after glucose load (2 g/kg) in rats, at first ½ h and up to 2 h period as compared with other groups extract glibenclamide on 8th, 15th and 22nd day, while aqueous and alcoholic extracts produced a significant reduction in FBGL. Glibenclamide (10 mg/kg) showed ($P < 0.005$) significant suppression in FBGL rise at first ½ h,

**Table 1:** Drugs and chemicals

| S.no | Materials | Company name |
|---|---|---|
| 1. | Alloxan | Quali Kems Fine Chem. Pvt, Ltd, Vadodara. |
| 2. | Methanol | ChangshuYangyuan Chemicals, China. |
| 3. | Alcohol | ChangshuYangyuan Chemicals, China. |
| 4. | Glibenclamide | Sanofi India Ltd, Ankleshwar. |

**Table 2:** Assessment of hypoglycemic activity on normal rats

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

**Table 3:** Assessment of antidiabetic activity in alloxan-induced diabetic rats

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

**Table 4:** Phytochemical screening of *A. racemosus*

| S.No. | Phytoconstituents | Aqueous | Alcoholic |
|---|---|---|---|
| 1. | Alkaloids | + | - |
| 2. | Carbohydrates | - | + |
| 3. | Glycosides | - | - |
| 4. | Phytosterols | + | - |
| 5. | Saponins | + | + |
| 6. | Fixed oils & Fats | - | - |
| 7. | Tannins & Phenolic compounds | + | + |
| 8. | Protein & Free amino acids | + | + |
| 9. | Gums & mucilage | - | - |
| 10. | Flavonoids | + | - |

1 h, and normalized FBGL within 2hr. The detailed results are summarized in Table 5.

## Antidiabetic Activity in Alloxan-Induced Diabetic Rats

FBGL in normal rats were in the range of 90–100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to a range of 252–266 mg/dl after 72 h. These values on subsequent days got stabilized by day 7 on an average of 255 mg/dl.

Changes in the FBGL in different groups are tabulated in Table 8. This data showed that the

**Table 5:** Effect of extracts of *A. racemosus* on fasting blood glucose level (FBGL) in normal rats

| Treatment | Dose (mg/kg) | Blood glucose level(mg/dl) | | |
|---|---|---|---|---|
| | | 7th day | 14th day | 21st day |
| Normal control | - | 82.21±5.58 | 80.29±6.53 | 82.64±5.28 |
| Glibenclamide | 10 | 71.59±2.41 | 65.36±3.80 | 61.54±2.54 |
| AQAR1 | 20 | 82.10±4.60 | 79.73±4.99 | 78.49±2.54 |
| AQAR2 | 30 | 84.10±5.14 | 81.57±6.99 | 80.45±5.04 |
| ALAR1 | 20 | 72.5±3.44 | 65.58±2.86 | 62.58±2.45 |
| ALAR2 | 30 | 73.36±2.67 | 68.51±2.92 | 65.45±1.76 |

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

**Table 6:** Effect of extracts of A. racemosus on 8th, 15, and 22nd day in normal rats

| Treatment | Dose (mg/kg) | Blood glucose level(mg/dl) | | |
|---|---|---|---|---|
| | | 8th day | 15th day | 22nd day |
| Normal control | - | 88.64±2.51 | 89.62±1.68 | 91.58±1.46 |
| Glibenclamide | 10 | 84.24±0.64 | 80.62±2.57 | 74.26±2.49 |
| AQAR1 | 20 | 80.17±2.18 | 76.28±1.45 | 72.84±5.81 |
| AQAR2 | 30 | 85.92±0.64 | 80.69±0.08 | 75.68±2.15 |
| ALAR1 | 20 | 84.62±1.85 | 78.52±2.64 | 70.42±0.46 |
| ALAR2 | 30 | 73.37±2.10 | 64.58±2.68 | 62.15±0.84 |

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

**Table 7:** Effect of extracts of *A. racemosus* on fasting blood glucose level (FBGL) in Alloxan-induced diabetic rats

| Treatment | Dose (mg/kg) | Blood glucose level (mg/dl) | | |
|---|---|---|---|---|
| | | 7th day | 14th day | 21st day |
| Normal control | - | 82.21±5.58 | 80.29±6.53 | 82.64±5.28 |
| Diabetic control | 10 | 368.16±10.9 | 398.20±12.45 | 412.58±13.46 |
| Glibenclamide | 10 | 314.15±16.04 | 287.32±19.02 | 221.30±14.69 |
| AQAR1 | 20 | 290.34±10.58 | 242.16±14.00 | 214.47±12.68 |
| AQAR2 | 30 | 294.15±12.45 | 272.36±11.57 | 248.16±16.04 |
| ALAR1 | 20 | 286.66±13.64 | 184.52±12.67 | 168.49±17.25 |
| ALAR2 | 30 | 240.25±16.02 | 224.60±14.62 | 196.31±9.64 |

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

**Table 8:** Effect of extracts of *A. racemosus on* 8th, 15th, and 22nd day in Diabetic rats

| Treatment | Dose (mg/kg) | Blood glucose level(mg/dl) | | |
|---|---|---|---|---|
| | | 8th day | 15th day | 22nd day |
| Normal control | - | 88.64±2.51 | 89.62±1.68 | 91.58±1.46 |
| Diabetic control | 10 | 356.20±16.53 | 374.00±12.48 | 382.41±6.03 |
| Glibenclamide | 10 | 258.6±18.49 | 154.48±15.64 | 148.36±14.62 |
| AQAR1 | 20 | 274.60±14.51 | 236.60±13.43 | 224.41±12.68 |
| AQAR2 | 30 | 280.96±15.62 | 264.25±10.62 | 230.29±10.80 |
| ALAR1 | 20 | 257.30±15.15 | 168.40±11.00 | 145.61±12.54 |
| ALAR2 | 30 | 328.90±14.59 | 309.50±9.07 | 284.35±8.57 |

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

blood glucose level of normal control animals has maintained throughout the study period.

The diabetic control group has shown a significant increase in FBGL during this 21st day study period. Glibenclamide (10 mg/kg) treated group has shown ($P < 0.05$) a significant decrease in FBGL during the 7th, 14th, and 21st day of the study period.

### Effect of *A. racemosus* Extracts on Anti-diabetic Activity in Alloxan-Induced Diabetic Rats

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

### OGTT on 8th, 15th, and 22nd Day

Both the aqueous and alcoholic extracts of *A. racemosus* are significantly ($P < 0.05$) suppress the rise in FBGL after glucose load (2 g/kg) in rats, at first 1/2 h and up to 2 h period as compared with other groups extract glibenclamide on 8th,15th and 22nd day, while aqueous and alcoholic extracts produced a significant reduction in FBGL. Glibenclamide (10 mg/kg) showed ($P < 0.05$) significant suppression in FBGL rise at first ½ h, 1 h and normalized FBGL within 2hr. The detailed results are summarized in Table 8.

## DISCUSSION

Many natural active compounds have been isolated from plants of different species. These active principles are complex carbohydrates, alkaloids, flavonoids, saponins, amino acids, steroids, peptides, terpenoids, 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 the gut, stimulating glycogenesis in the liver, and/or increasing glucose utilization by the body. These compounds may also exhibit antioxidant, hypolipidemic, anticataract activities, and restore enzymatic functions, repair and regeneration of pancreatic islets, alleviation of liver and renal damage.

Crude aqueous and alcoholic extracts of leaves of *A. racemosus* at a dose of 20 and 30 mg/kg showed a significant effect on the glucose tolerance of rats and it also showed a reduction in the FBGL of the normoglycemic rats, thus revealing the hypoglycemic nature of the extracts. The effect was more pronounced for both extracts. These findings indicate that the extracts might be producing the hypoglycemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production[12] or by the inhibition of intestinal glucose absorption.

Phytochemical analysis of extracts of leaves of *A. racemosus* revealed the presence of secondary metabolites that have been shown to possess an antidiabetic effect in other plants. Saponins, alkaloids, and flavonoids 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.

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 a 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 the 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 of treatment. The difference observed between the initial and final fasting serum glucose levels of extract treated hyperglycemic rat's revealed the antihyperglycemic effect of leaves of *A. racemosus* throughout the study. The effect of the extracts was compared to that of the reference standard, glibenclamide and was found to be significant.

Extracts of leaves of *A. racemosus* appear to be attractive materials for further studies leading to possible drug development for diabetes. The 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 *A. racemosus* in normoglycemic rats and alloxan-induced diabetic rats and the results reveal that the plant has beneficial effects on blood glucose levels. In the current scenario, herbs are the potent sources of medicines used in the treatment of various diseases and disorders. Since plants are used as medicine there is a prompt need for evaluation of plant species; therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of leaves of *A. racemosus*. The Phytochemical evaluation has revealed the presence of alkaloids, terpenoids, saponins, flavonoids, phenols, and tannins.

The aqueous and alcoholic extracts had hypoglycemic activity because of the presence of flavonoids which are rich in the treatment of hypoglycemia with fewer side effects. Flavonoids might be producing the hypoglycemic effect by a mechanism independent from insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. The present study *A. racemosus* of both aqueous and alcoholic extracts was showed a significant effect on glucose tolerance and also showed a reduction in FBGL in normal diabetic rats.

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

Results of antidiabetic 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 a significant reduction in blood glucose levels in normal and alloxan-induced diabetic rats and produced maximum antidiabetic activity and are higher than the hypoglycemic activity of glibenclamide in the diabetic rats. In glucose loaded animals, the drug has reduced the blood glucose to normal levels. The drug may be

acting by potentiating the pancreatic secretion or increasing the glucose uptake. In conclusion, these extracts showed a significant antidiabetic 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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