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Research



Pharmacological Screening And Phytochemical Evaluation Of Anti-Diabetic Activity Of *Asparagus Racemosus* Leaves In Normal And Alloxan Induced Diabetic Rats

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	<h3>Abstract</h3>
<p>Published on: 07 Nov 2024</p>	<p>Diabetes mellitus is a most common endocrine disorder, affecting more than 300 million people worldwide. For 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. In order to identify complementary or alternative approaches to existing medications, we studied the anti-diabetic potential of leaves of <i>A.Racemosus</i>. The acute oral toxicity studies of the extracts revealed no toxic effects up to the levels of 2000mg/kg b.wt. The aqueous and alcoholic extracts of 20 and 30mg/kg body weight of <i>A.Racemosus</i> was screened for the presence of hypoglycemic and antidiabetic activity. In this study diabetes was induced by a single IP dose Alloxan monohydrate in 72hrs fasted rats. The FBGL was carried on 7th, 14th and 21st day and OGTT was measured on 8th, 15th and 22nd day. Glibeclamide was taken as the standard and the results are quite comparable with it. The studies were indicated that the leaves of <i>A.Racemosus</i> are effective in regeneration of insulin secreting β-cells and thus possess antidiabetic activity. The aqueous and alcoholic extracts showed significant effect in decreasing the Fasting blood Glucose level and oral glucose tolerance test of rats and it's also showed good hypoglycemic activity in normal glycemic rats. The preliminary phytochemical analysis of the extracts of <i>A.Racemosus</i> revealed the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, phenolics and glycoside as the possible biologically active principles.</p>
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INTRODUCTION

Diabetes Mellitus (DM)

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 in all age groups, the general public has a concern about its control and treatment¹.

Classification of DM

Diabetes is classified by underlying cause. The most common forms of diabetes are categorized as

Type 1, or insulin-dependent diabetes mellitus (IDDM) - an autoimmune disease in which the body's own immune system attacks the pancreatic beta cells, rendering it unable to produce insulin and

Type 2, or non-insulin-dependent diabetes mellitus (NIDDM) - in which there is resistance to the effects of insulin or a defect in insulin secretion.

Type 2 diabetes commonly occurs in adults associated with obesity. There are many underlying factors that contribute to the high blood glucose levels in these individuals. An important factor is the resistance to insulin in the body essentially ignoring its insulin secretions. A second factor is the decreased production of insulin by the cells of the pancreas. Therefore, an individual with Type 2 diabetes may have a combination of deficient secretion and deficient action of insulin. In contrast to Type 2 diabetes, Type 1 diabetes most commonly occurs in children and is a result of the body's immune system attacking and destroying the beta cells. The trigger for this autoimmune attack is not clear, but the result is the end of insulin production².

Multiple risk factors for the development of Type 2 diabetes mellitus³:

- Family history (parents with diabetes).
- Obesity (i.e., $\geq 20\%$ over ideal body weight or body mass index $\geq 25\text{kg/m}^2$).
- Habitual physical inactivity.
- Impaired glucose tolerance.
- Hypertension ($\geq 140/90\text{mm Hg}$ in adults).
- High density lipoprotein (HDL) cholesterol $\leq 35\text{mg/dl}$ and/or triglyceride level $\geq 250\text{mg/dl}$.

History

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 diabetics⁵.

Historical accounts reveal that as early as 700-200 BC, diabetes mellitus was a well recognized disease in India and was even distinguished as two types, a genetically based disorder and other 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.

Epidemiology⁶

Present status projects that incidence of diabetes is on rise. Present number of diabetics worldwide is 150 million and according to new estimates from researchers at the World Health Organization (WHO), there will be an increase of about 300 million or more by the year 2030 (Warner, 2004). Only in year 2001, about 441,004 deaths were registered and 49,855 of them provoked by diabetes, representing 11.2% of the total population. In 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.

Sugar Regulation: Carbohydrate, protein and lipid metabolism⁸

Glucose is an essential fuel for the body and is the main source of energy for the tissue cell. The amount of glucose in the blood is controlled mainly by the hormones insulin and glucagon. The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Glucose is transported into the beta cell by Type 2 glucose transporters (GLUT2). As glucose metabolism proceeds, ATP is produced which closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell. The rise in positive charge inside the beta cell causes depolarization thereby opening the voltage-gated calcium channels and allowing calcium ions (Ca^{2+}) to flood into the cell. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

SIGNS AND SYMPTOMS¹¹

In both the types of diabetes, signs and symptoms are more likely to be similar as the blood sugar is high, either due to less or no production of insulin, or insulin resistance. In any case, if there is inadequate glucose in the cells, it is identifiable through certain signs and symptoms. These are quickly relieved once the diabetes is treated and also reduce the chances of developing serious health problems.

Type 1 Diabetes

In type 1 the pancreas stops producing insulin due to autoimmune response or possibly viral attack on pancreas. In absence of insulin body cells don't get the required glucose for producing ATP (Adenosine Triphosphate) units which results into primary symptom in the form of nausea and vomiting. In later stage, which leads to ketoacidosis, the body starts breaking down the muscle tissue and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advance stages, coma and death is witnessed.

Type 2 Diabetes

Increased fatigue: due to inefficiency of the cell to metabolize glucose, reserve fat of body is metabolized to gain energy. When fat is broken down in the body, it uses more energy as compared to glucose; hence body goes in negative calorie effect, which results in fatigue.

Polydipsia: As the concentration of glucose increases in the blood, brain receives signal for diluting it and, in its counteraction we feel thirsty.

Polyuria: Increase in urine production is due to excess glucose present in body. Body gets rid of the extra sugar in the blood by excreting it through urine. This leads to dehydration because along with the sugar, a large amount of water is excreted out of the body.

Polyphagia: The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar levels in blood, body produces insulin which leads to increased hunger.

Weight fluctuation: Factors like loss of water (polyuria), glucosuria, metabolism of body fat and protein may lead to weight loss. Few cases may show weight gain due to increased appetite.

Blurry vision: Hyperosmolar, hyperglycaemia, nonketotic syndrome is the condition when body fluid is pulled out of tissues including lenses of the eye; this affects it's to focus, resulting blurry vision.

Irritability: It is a sign of high blood sugar of the inefficient glucose supply to the brain and other body organs, which make us, feel tired and uneasy.

Infections: The body gives few signals whenever there is fluctuation in blood sugar(due to suppression of immune system) by frequent skin infections like fungal or bacterial or UTI(urinary tract infection).

Poor wound healing: High blood sugar resists the flourishing of WBC, (white blood cell) which is responsible for body immune system. When these cells do not function accordingly, wound healing is not at good pace. Secondly, long standing diabetes leads to thickening of blood vessels which affect proper circulation blood in different body parts.

Pathophysiology¹³:

Diabetes occurs when there is a dis-balance between the demand and production of the hormone insulin.

Control of blood sugar

When food is taken, it is broken down into smaller components. Sugars and carbohydrates are thus broken down into glucose for the body to utilize them as an energy source. The liver is also able to manufacture glucose. In

normal persons the hormone insulin, which is made by the beta cells of the pancreas, regulates how much glucose is in the blood. When there is excess of glucose in blood, insulin stimulates cells to absorb enough glucose from the blood for the energy that they need. Insulin also stimulates the liver to absorb and store any excess glucose that is in the blood. Insulin release is triggered after a meal when there is a rise in blood glucose. When blood glucose levels fall, during exercise for example, insulin levels fall too. High insulin will promote glucose uptake, glycolysis (break down of glucose), and glycogenesis (formation of storage form of glucose called glycogen), as well as uptake and synthesis of amino acids, proteins, and fat.

Normal Responses to Eating and Fasting¹⁴

1. In a fed state: there is increased insulin secretion, causing Glycolysis, glycogen storage, fatty acid synthesis/storage, and protein synthesis.
2. After an overnight fast: there is low insulin and high glucagon that can cause glycogen breakdown, hepatic Gluconeogenesis, and Lipolysis.
3. After a prolonged fast: there is extremely low insulin and low glucagon, this causes lipolysis to take over. Lipids are the main fuel source. Gluconeogenesis is minimized, as it causes nitrogen wasting, ammonia build-up, and loss of muscle mass.

Hormones

Hormones that raise blood sugar include glucagon, epinephrine and norepinephrine, cortisol, Growth hormone etc. These hormones are released due to *stress*. Thus during phases of stress like an infection, surgery or pregnancy diabetes control worsens and blood sugar rises.

MATERIALS AND METHODS

The designing of methodology involves a series of steps taken in a systematic way in order to achieve the set goal(s) under the prescribed guidelines and recommendations. It includes in it all the steps from 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 good build of mind and a good and soft technical hand to handle the materials and procedure in a true scientific manner.

Drugs and Chemicals

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

Table 1: Drugs and Chemicals

S.No	Materials	Company Name
1.	Alloxan	Quali Kems Fine Chem Pvt, Ltd, Vadodara.
2.	Methanol	Changshu Yangyuan Chemicals, China.
3.	Alcohol	Changshu Yangyuan Chemicals, China.
4.	Glibenclamide	Sanofi India Ltd, Ankleshwar.

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 and the room temperature was maintained at 27° C. Animal studies had approval of IAEC.

Plant Material Collection

The leaves of *A.racemosus* was collected from the local market in Hyderabad in the month of January and was identified and authenticated from 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 extraction process.

Preparation of plant extracts

Preparation of Aqueous Extract

Dried leaves of *A.racemosus* were taken about 20gms into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled upto 80-90°C for 4-5hrs. Further the extract was filtered with whatmann 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 further experiment to check the activities.

Preparation of Alcoholic Extract

Dried leaves of *A.racemosus* were taken about 20gms into 250ml beaker containing 200ml of Alcohol. The contents were mixed well and then the mixture was boiled upto 50-60°C for 4-5hrs. Further the extract was filtered with whatmann 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 further experiment to check the activities.

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.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Triterpenoids

Salkowski's Test: The extracts were treated with chloroform and filtered separately. The filtrate was treated with few drops of concentrated sulphuric 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

Froth Test: The extracts were diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 mins. The formation of 1 cm layer of foam indicates the presence of saponins.

Lieberman Burchard Test: The extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride boiled and cooled. Concentrated sulphuric acid was added through the sides of test tube. The formation of brown ring at the junction indicated the presence of steroidal saponins.

Flavonoids

Alkaline reagent Test: The extracts were treated with few drops of sodium hydroxide separately. Formation of intense yellow colour less on addition of few drops of dilute acid indicates the presence of flavonoids.

Lead acetate Test: The extracts were treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Phenolic and Tannins

Ferric chloride Test: The extract was treated with few drops of neutral ferric chloride solution. The formation of bluish black colour indicates the presence of phenolics nucleus.

Gelatin Test: To the extract, 1%gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins.

Vanillin hydrochloride Test: the extracts were treated with few drops of vanillin hydrochloride reagent. The conformation of pinkish red colour indicates the presence of tannins.

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of 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 dose of 2000mg/kg body weight.

Pharmacological evaluation

Preparation of extracts

The aqueous and alcoholic extracts of *A.racemosus* suspended in water in presence of 3%v/v Tween-80 solution.

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 *A.racemosus* 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 2: Group Classification

Group	Treatment	Dose(mg/kg)
Group 1	Normal control received distilled water	10ml/kg
Group 2	Standard group received Glibenclamide	10ml/kg
Group 3	Aqueous extract of <i>A.racemosus</i>	20 mg/kg
Group 4	Aqueous extract of <i>A.racemosus</i>	30 mg/kg
Group 5	Alcoholic extract of <i>A.racemosus</i>	20 mg/kg
Group 6	Alcoholic extract of <i>A.racemosus</i>	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 0th, 7th, 14th and 21st 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 (1st, 8th, 15th and 22nd 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 1st, 8th, 15th and 22nd 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 16 hour fasted rats by 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 next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than 200 mg/dl were selected and used for further studies

All the animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 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.

Table 3: Group Classification

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

Effect of Aqueous and Alcoholic extracts of *L.sativum* 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 7th, 14th and 21st 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 8th, 15th and 22nd 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 \pm 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.

RESULTS

Phytochemical screening of *A.racemosus*.

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

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	+	-

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

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 7th, 14th and 21st 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: 10

Repeated administration of both aqueous and alcoholic extracts had significantly ($p < 0.005$) reduced the FBGL on 7th, 15th and 21st day, indicating these extracts can produce hypoglycemia on repeated administration. However hypoglycemic activity was more significant on 7th, 14th and 21st 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 Table 5.

Effect of extracts of *A.racemosus* on fasting blood glucose level (FBGL) in normal rats**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)		
		7 th day	14 th day	21 st 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 Vs all groups. Parent thesis indicates % reduction in BGL.

Oral glucose tolerance test (OGTT)

Both the aqueous and alcoholic extracts of *A.racemosus* significantly ($P < 0.005$) 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 glibenclamide on 8th, 15th and 22nd 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 6.

Table 6: Effect of extracts of *L.sativumon* 8th, 15th and 22nd day in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level(mg/dl)		
		8 th day	15 th day	22 st 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 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 21st day study period. Glibenclamide (10mg/kg) treated group has shown (p<0.05) significant decrease in fasting blood glucose level during 7th, 14th and 21st day of study period.

Effect of *A.racemosus* 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 7th, 14th and 21st 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 Table 7.

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)		
		7 th day	14 th day	21 st 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 Vs all groups. Paranthesis indicates % reduction in BGL.

Oral glucose tolerance test (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 (2g/kg) in rats, at first half-an-hour and upto 2hr time period as compare with other groups extract glibenclamide on 8th, 15th and 22nd 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 Table 8.

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)		
		8 th day	15 th day	22 st 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 Vs all groups. Paranthesis indicates % reduction in BGL.

DISCUSSIONS

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 authors⁷³⁻⁷⁶. 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 diabetes⁷⁷. 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 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 activities⁷⁸. These effects might be achieved by facilitating insulin release from pancreatic β -cells, inhibiting glucose absorption in gut, stimulating glycogenesis in liver and/ or increasing glucose utilization by the body. These compounds may also exhibit antioxidant, hypolipidemic and anticataract activities, and restore enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage⁷⁹.

Crude aqueous and alcoholic extracts of leaves of *A.racemosus* 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 production⁸⁰ or by the inhibition of intestinal glucose absorption⁸¹.

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^{82,83}. 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 *A.racemosus* 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 *L.sativum* revealed the presence of secondary metabolites that have been shown to possess 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. Extracts of leaves of *A.racemosus* 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 *A.racemosus* 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 *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 the presence of flavonoids which are rich in treatment of hypoglycaemia with less side effects. Flavonoids might be producing hypoglycaemic 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 significant effect on glucose tolerance and also showed reduction in fasting blood glucose levels in normal diabetic rats.

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 22nd day which was evident from the 7th day onwards as compared to standard. The aqueous and alcoholic extract of *A.racemosus* 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.

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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