

ANTIDIABETIC EFFECT OF ETHYL ACETATE EXTRACT OF *ASPARAGUS RACEMOSUS* WILLD ON HIGH –FAT DIET AND STREPTOZOTOCIN INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Background: Diabetes is a costly disease with high financial burden. This study is to treat the diabetes using natural resources. Therefore, the present study was designed to investigate the ethyl acetate extract of *Asparagus racemosus* Willd for alleviating the symptoms of diabetes mellitus. **Methods:** After induction of the diabetic rat model T2DM using high fat diet (HFD)/streptozotocin (STZ), thirty male Wistar albino rats were assigned into normal control, T2DM untreated, and ethyl acetate extract of *Asparagus racemosus* Willd treated diabetic rat groups. T2DM induction through 3 weeks of high fat diet (HFD) intervention was followed by single low dosage of STZ (30mg/kg dissolved in 0.1 mol/L citrate buffer at pH 4.5, i.p). **Result:** Diabetic rat models showed a significant increase in blood glucose level as compared to the treated animals, which were determined by OGTT. T2DM rats have decreased levels of liver glucose 6 phosphate dehydrogenase (G6PD), reduced glutathione (GSH), nitric oxide (No),

and antioxidant enzymes. Furthermore, the present study showed hypoglycemic, hypolipidemic, and antioxidant activity of the ethyl acetate extract of *Asparagus racemosus* Willd as confirmed by its ability for ameliorating most of the alteration caused in the study parameters of diabetic rats. **Conclusion:** The ethyl acetate extract of *Asparagus racemosus* Willd may be useful as therapy against oxidative stress and liver damage in type 2 diabetes mellitus and is therefore recommended for further studies.

KEYWORDS: T2DM, Ethyl acetate extract of *Asparagus racemosus* Willd, hyperglycemia, Lipid profile, Liver toxicity, Oxidative stress.

INTRODUCTION

It is a metabolic disorder characterized by hyperglycaemia, glycosuria, hyperlipaemia, negative nitrogen balance and sometimes ketonaemia. Several pathogenic processes are involved in the development of diabetes.^[1] These range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia. About 3.4 million peoples died from consequences of high fasting blood sugar during 2004. About 347 million people worldwide have diabetes. The death rate of diabetes mellitus is more in poor countries. About 80% of diabetes deaths occurs in low and middle income countries. According to World health organization, diabetes will be the 7th leading cause of death by 2030 and it will double between 2005 and 2030. There are several ongoing studies focused to prevent and delay the onset of diabetes mellitus but the complete cure for diabetes is not reported yet through synthetic medicines. There are several medicinal plant approaches towards the treatment and prevention of diabetes mellitus, recently many herbal related studies are going on successfully for the treatment and prevention of diabetes mellitus.^[2-3] Type 2 diabetes (formerly called non-insulin-dependent or adult-onset) results from the body's ineffective use of insulin. Type 2 diabetes comprises 90% of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity. Symptoms may be similar to those of Type 1 diabetes, but are often less marked. Until recently, this type of diabetes was seen only in adults but it is now also occurring in children. Many traditional plants have been reported in India for diabetes, but only a small number of these have received scientific and medical evaluation to assess their efficacy. On the basis of ethno medical/tribal information, *Asparagus racemosus* willd has been used to treat and prevent diabetes. *Asparagus racemosus* willd possess a diverse number of pharmacological activities including free radical scavenging activity.^[4-6] anticholinesterase activity^[7,8] and anti-inflammatory property. However, the studies on anti-diabetic effects of *Asparagus racemosus*

willd extracts were not focused on the enzyme inhibitory activity thus, the present study is designed to evaluate the in-vivo anti-diabetic activity of ethyl acetate extract of *Asparagus racemosus* willd and to understand how the extract acts against high –fat diet and streptozotocin induced diabetes.

MATERIALS AND METHODS

Identification of Plant Materials

The root part of the plant *Asparagus racemosus* willd Family Asparagaceae was collected from the forests of Doddabetta in Nilgiris. The plant species was identified and authenticated by the Dr.S. Rajan, PhD., Field Botanist, Survey of Medicinal Plants and Collection Unit, Department of AYUSH, Ministry of Health and Family Welfare, Govt of India. They deposited in the herbarium of the Department of Pharmacology, JSS College of Pharmacy, Ooty. The leaves of the plant were used in the present study.

Chemicals

All the chemicals used in the study are of analytical grade and are purchased from Sigma chemicals.

Preparation of crude extract

The medicinal root part of the plant (*Asparagus Racemosus* Willd) was sun dried and ground to a coarse powder and stored in an air tight container. This coarse powder was subjected to successive extraction with ethyl acetate (77°C) by continuous soxhlation. After collection of extracts, it is kept at temperature 37°C until solvent is completely evaporated and finally dried in desiccator.

Animals

Healthy Male Wistar rats, weighing 180-220g, were procured from the animal house, JSS College of Pharmacy, Ootacamund, India. The animal house was well ventilated and animals had 12 ± 1 h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period and room temperature was maintained at 25 ± 1°C. The animals were fed with standard rat feed and water ad libitum. The experiments were conducted as per the guidelines of CPCSEA, Chennai, India (approval no. JSSCP/IAEC/PH./COLOGY-06/2016-17).

Induction of type 2 diabetes in rats

The rats were allocated into dietary regimens by feeding HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks.^[9] The composition and preparation of HFD were as described elsewhere. After the 4 weeks of dietary manipulation, the group of rats fed by HFD were injected intraperitoneally (i.p.) with low dose of STZ (30 mg/kg), while the respective control rats were given vehicle citrate buffer (pH 4.5) in a dose volume of 1mL/kg, i.p. The fasting blood glucose [FBG] was measured 72 hours after the STZ injection. The rats with the FBG of more than 300 mg/dL were considered diabetic and selected for further pharmacological studies. The rats were allowed to continue to feed on their respective diets until the end of the study.

Composition of high fat diet

S.No.	Ingredients	Diet g/kg
1	Powdered NPD	365
2	Lard	310
3	Casein	250
4	Cholesterol	10
5	DL-methionine	03
6	Yeast powder	01
7	Sodium chloride	01

Experimental design

In this experiment, a total of 30 rats (6 normal; 24 STZ-induced diabetic rats) were used and were divided into five groups of 6 rats in each. Group I: normal control rats administered saline (NC); Group II: Diabetic control rats administered saline (DC); Group III: Diabetic + Glibenclamide (10mg/kg) (DG); Group IV: Diabetic + EEAR(200mg/kg)(DEEAR); Group V: Diabetic + EEAR(400mg/kg) (DEEAR); All the treatment groups were administered orally for 21 days. Body weight, fluid intake, food intake and glucose levels were analyzed every week, Lipid and lipoprotein profile (TC, TG, HDL-c, LDL-c, VLDL-c) was analyzed in serum after 21 days.[10] On the day of termination of the study, organs such as liver and kidney were isolated, weighed and stored. Liver was used for the assay of hepatic glucose-6-phosphatase and glycogen content. Antioxidant parameters were assessed in both liver and kidney.

OGTT in diabetic rats

Prior to an OGTT, diabetic rats were fasted for 16 h. Extracts and the reference drug, glibenclamide were orally administered to two groups of 6 rats each respectively. Thirty minutes later, glucose (3 g/kg) was orally administered to each rat with a feeding syringe. Blood samples were collected from the tail vein by tail milking at, 0 (just before the oral administration of glucose), 30, 60, 120 and 180 min after glucose load for the assay of glucose.^[11]

Assay of glucose-6-phosphatase in liver

The hepatic glucose-6-phosphatase activity was assayed by the method of Baginski et al., 1974.^[12] Here, the glucose-6-phosphate in the liver extract is converted into glucose and inorganic phosphate. The inorganic phosphate liberated is determined with ammonium molybdate; ascorbic acid is used as the reducing agent. Excess molybdate is removed by the arsenite citrate reagent, so that it can no longer react with other phosphate esters or with inorganic phosphate formed by acid hydrolysis of the substrate. Arsenite-citrate also stabilizes the system. The amount of phosphate liberated per unit time, determined as the blue phosphomolybdous complex at 700 or 840 nm, is a measure of the glucose-6-phosphatase activity. The glucose-6-phosphatase activity was expressed as mmol of phosphate released /min/ mg of protein.

Assay of liver glycogen content in liver

The liver glycogen content was determined by the enzymatic method of Murat and Serfaty, 1974.^[13] Liver was homogenized with the Potter-Elvehjem homogenizer in ice-cold citrate buffer (0.1 mol/liter, pH 4.2). Immediately after homogenization, 10 ml of the mixture was used to determine free glucose in the tissue by the glucose-oxidase method, according to Trinder, 1969. Amyloglucosidase dry powder was then mixed with the homogenate and allowed to stand at room temperature for 2 h. A 10 mL sample was used to determine total glucose. Initial free glucose was subtracted from the total glucose, and the resultant value was used to calculate the glycogen content. The glycogen content was expressed as mg/g wet tissue.

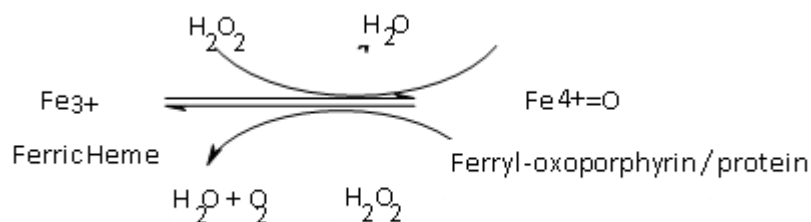
Estimation of antioxidant parameters**Preparation of tissue homogenate**

One part of the tissue such as liver and kidney were homogenized with 0.1 M tris-HCl buffer (pH 7.4) in Elvenjan homogenizer fitted with a teflon plunger at 600 rpm for 30 min. The

homogenate was centrifuged at 2000 rpm for 10 min at 4 °C and the supernatants were used for *in vivo* antioxidant activity.

Estimation of CAT

Catalase is an enzyme present in the cells of plants, animals and aerobic (oxygen requiring) bacteria. Catalase is located in a cell organelle called the peroxisome. In animals, catalase is present in all major body organs. The role of catalase is to scavenge hydrogen peroxide and prevent oxidative damage in the cell. Catalase is a heme containing protein that can convert hydrogen peroxide to water and oxygen in two-step reaction cycle. In the first step, one molecule of hydrogen peroxide is converted to water. The catalytic cycle begins with the oxidation of the ferric heme by two electrons by hydrogen peroxide to form the ferryl-oxo porphyrin/protein radical intermediate known as compound 1. The catalase cycle is completed by the reduction of compound 1 to the ferric enzyme by hydrogen peroxide, resulting in production of molecular oxygen (Li and Goodwin, 2004).



Chemicals and reagents

Hydrogen peroxide (7.5 mM): 1.043 ml of 30% w/w H₂O₂ was made up to 100 mL with sodium chloride and EDTA solution (9 g of NaCl and 29.22 mg of EDTA dissolved in one litre distilled water). Potassium phosphate buffer (65 mM, pH 7.8): 2.2 g of potassium dihydrogen phosphate and 11.32 g of dipotassium hydrogen phosphate were dissolved in 250 ml and 1 L distilled water, respectively and mixed together. The pH was adjusted to 7.8 with KH₂PO₄. Sucrose solution: 10.95 g of sucrose was dissolved in 100 mL of distilled water.

Procedure

2.25 mL of potassium phosphate buffer (65 mM, pH 7.8) and 100 µL of the tissue homogenate/ sucrose (0.32 M) were incubated at 25° C for 30 min. 0.65 mL of H₂O₂ (75 mM) was added to initiate the reaction. The change in absorption at 240 nm was measured for 2-3 min, and dy/dx for 1 min for each assay was calculated and the results are expressed as CAT units / mg of tissue (Beers and Seizer, 1952).

$$\text{CAT (U) / 100 } \mu\text{L of Sample} = \frac{[(dy/dx) \times 0.003]}{[38.3956 \times 10^{-6}]}$$

The dy/dx (change in absorbance/min) was calculated for each assay divided by 38.3956×10^{-6} (molar extinction coefficient of H_2O_2 at 240 nm) to obtain $\mu\text{M/l}$ of H_2O_2 converted to H_2O per min, multiplied by 0.003 to obtain micromoles. H_2O_2 converted to H_2O per min in 3 ml by 0.1 ml sample.

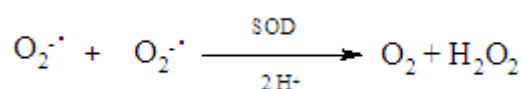
SOD estimation

One of the most effective intracellular enzymatic antioxidants is superoxide dismutase (SOD). Superoxide dismutase is the antioxidant enzyme that catalyzes the dismutation of $\text{O}_2^{\cdot-}$ to O_2 and to the less reactive species H_2O_2 . While this enzyme was isolated as early as 1939, it was only in 1969 that McCord and Fridovich proved the antioxidant activity of SOD [14].

Superoxide dismutase exists in several isoforms, differing in the nature of active metal centre and amino acid constituency, as well as their number of subunits, cofactors and other features. In humans there are three forms of SOD: cytosolic Cu, Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD). SOD destroys $\text{O}_2^{\cdot-}$ with remarkably high reaction rates, by successive oxidation and reduction of the transition metal ion at the active site in a “Ping-Pong” type mechanism.^[15]

Under physiological conditions, a balance exists between the level of reactive oxygen species (ROS) produced during normal cellular metabolism and the level of endogenous antioxidants, which serve to protect tissues from oxidative damage.

Disruption of this balance either through increased production of ROS or decreased levels of antioxidants produce a condition known as oxidative stress and leads to variety of pathological conditions. To protect against oxidative damage, organisms have developed a variety of antioxidant defenses that include metal sequestering proteins, use of compounds such as vitamin C, E and specialized antioxidant enzymes. One family of antioxidant enzymes, the superoxide dismutases (SOD) functions to remove damaging ROS from the cellular environment by catalyzing the dismutation of two superoxide radicals to hydrogen peroxide and oxygen.^[16]



SOD measurement was carried out on the ability of SOD to inhibit spontaneous oxidation of epinephrine to adrenochrome.

Chemicals and reagents

Sodium carbonate buffer (0.05 M, pH 10.2): 5.3 g of sodium carbonate and 1.2 g of sodium bicarbonate were dissolved separately in 1:1 of distilled water, which served as a stock solution. Buffer was prepared by mixing 64 mL of sodium carbonate and 70 mL of sodium bicarbonate solutions. The pH of the buffer was adjusted to 10.2 using the above stock solution accordingly.

Adrenaline (9 mM): 0.03 g of adrenaline was dissolved in distilled water and the final volume was made up to 10 mL with distilled water containing a drop of concentrated HCl (to bring pH down to 2). Adrenaline being sensitive, the vial was kept covered with aluminum foil at all times.

Sucrose (0.3199 M) solution: 10.96 g of sucrose was dissolved in distilled water and the volume was made up to 100 mL.

Procedure

2.8 mL of sodium carbonate buffer (0.05 mM) and 0.1 mL of tissue homogenate or sucrose (blank) was incubated at 30 °C for 45 min. The absorbance was then adjusted to zero to sample. Thereafter the reaction was initiated by adding 10 µL of adrenaline solution (9 mM). The change in absorbance was recorded at 480 nm for 8-12 min. Throughout the assay, the temperature was maintained at 30 °C. Similarly, SOD calibration curve was prepared by taking 10 unit/mL as standard solution. One unit of SOD produced approximately 50% inhibition of auto-oxidation of adrenaline. The results were expressed as unit (U) of SOD activity/mg of tissue.^[17]

Thiobarbituric acid reactive substances (TBARS) estimation

Lipid peroxidation is commonly regarded as a deleterious process leading to structural modification of complex lipid protein assemblies, such as biomembranes and lipoproteins, and is usually associated with cellular malfunction. During lipid peroxidation, a polar oxygen moiety is introduced into the hydrophobic tails of unsaturated fatty acids. This process is of dual consequence: the presence of hydroperoxy group disturbs the hydrophobic lipid/lipid and lipid/protein interactions, which leads to structural alterations of biomembranes and

lipoproteins; hydroperoxy lipids are sources for the formation of free radicals. When free radicals are generated, they can attack polyunsaturated fatty acids in cell membrane leading to a chain of chemical reactions called lipid peroxidation. As the fatty acid is broken down, the hydrocarbon gases and aldehyde are formed. The most common method used to assess malondialdehyde (MDA) is the thiobarbituric acid assay.^[18]

Chemicals and reagents

Thiobarbituric acid solution: 0.8 g of thiobarbituric acid was dissolved in distilled water and volume was made up to 100 mL. The pH was adjusted to 7.4 with 1 N NaOH / 0.1 N HCl solution.

Acetic acid solution: 20 mL of acetic acid was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The pH was adjusted to 3.5 with 1 N NaOH/0.1 N HCl solution.

Sodium lauryl sulfate solution: 8.1 g of sodium lauryl sulfate was dissolved in distilled water and the volume was made up to 100 mL with distilled water.

Mixture of n-butanol and pyridine (15:1 v/v): 15 ml of n-butanol and 1 ml of pyridine were mixed together.

Reagents

Thiobarbituric acid solution: Thiobarbituric acid (0.8 g) was dissolved in distilled water and volume was made up to 100 ml with distilled water. The pH was adjusted to 7.4 with 1 N NaOH/0.1 N HCl solution.

Acetic acid solution: Glacial acetic acid, (20 ml) was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The pH was adjusted to 3.5 with 1 N NaOH/0.1 N HCl solution.

Sodium lauryl sulfate solution: Sodium lauryl sulfate (8.1 g) was dissolved in distilled water and the volume was made up to 100 mL with distilled water.

Mixture of n-butanol and pyridine (15:1 v/v): n-butanol (15 mL) and 1 mL of pyridine were mixed together.

Procedure

To 1 mL of tissue homogenate, 0.2 ml of sodium lauryl sulfate solution (8.1%), 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of thiobarbituric acid solution (0.8% w/v, pH 7.4) were added. This incubation mixture was made up to 5.0 mL with double distilled water and then heated in boiling water bath for 30 min. After cooling, the red chromogen was extracted with 5 mL of the mixture of n-butanol and pyridine (15:1 v/v) and centrifuged at 4000 rpm for 10 min. The organic layer was taken and its absorbance was measured at 532 nm. The results were expressed as nM of MDA/mL for serum and nM of MDA/mg for tissue using molar extension co-efficient of the chromophore ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$).^[19]

$$\begin{array}{l} \text{nM of MDA/mg of tissue} \\ \text{or ml of serum} \end{array} = \frac{\text{OD} \times \text{volume of homogenate} \times 100 \times 10^3}{1.56 \times 10^5 \times \text{volume of extract taken}}$$

RESULTS AND DISCUSSION

Effect of extract on OGTT

The results of OGTT in diabetic rats are given in Table 1. The data reveal that the extract cause a significant decrease in the blood glucose level at 90 min after oral administration to HFD and STZ-diabetic rats. The plant root ethyl acetate extract of the *Asparagus racemosus* will produce a significant attenuation ($p < 0.001$) in the blood glucose level at 120 min to 180 min when compared with the diabetic control.

Effect of extracts on blood glucose level

The results of the blood glucose level are given in Table 2. The data reveal that the extracts and fractions cause significant ($p < 0.001$) time-dependent decrease in the blood glucose level after oral administration at 7, 14 and 21 days when compared to the diabetic control group. After 21 days of daily treatment with the plant root ethyl acetate extract of the *Asparagus racemosus* will (200 and 400 mg/kg) results show significant dose-dependent fall in blood glucose levels by 39, 53, 42 and 53%, respectively. Glibenclamide (10 mg/kg) treated rats also show significant decrease in the blood glucose level (51 %).

Effect of extract on body weight, fluid and food intake

The results of body weight, fluid and food intake are given in Table 3. The data reveal that the normal control rats show an increase in body weight from 290.83 ± 0.54 to 339 ± 0.36 g. The diabetic control rats also show an increase in body weight from 284.33 ± 0.61 to 316.50

± 0.43 g. The plant root ethyl acetate extract of the *Asparagus racemosus* will (200 and 400 mg/kg) treated diabetic rats show gradual increase in body weight from 286.50 ± 0.50 to 324.50 ± 0.62 g and 273.67 ± 1.13 to 315.50 ± 0.43 g, respectively.

In chronic treatment both the extract show a significant decrease in the fluid and food intake as compared to diabetic control ($p < 0.001$).

Effect of extract on glucose-6-phosphatase and liver glycogen content

The results of glucose-6-phosphatase and liver glycogen content are given in Table 4. The data reveal that the glucose-6-phosphatase activity in the liver show significant decrease ($p < 0.001$, $p < 0.01$) in the plant root ethyl acetate extract of the *Asparagus racemosus* will when compared to the diabetic control group. Similarly, liver glycogen content also show a significant increase ($p < 0.001$, $p < 0.01$) in the extract when compared to the diabetic control group.

Effect of extract on lipid and lipoproteins

The results of lipid and lipoproteins are given in Table 5. The data reveal that the diabetic animals show a significant increase in the level of TC, TG, LDL and VLDL cholesterol and a decrease in the level of HDL cholesterol in serum, when compared to the normal animals ($p < 0.001$). The levels of TG, TC, LDL and VLDL significantly decreases ($p < 0.001$) whereas HDL significantly increases ($p < 0.001$) in the plant root ethyl acetate extract of the *Asparagus racemosus* will when compared to the diabetic control group.

Effect of extract on antioxidant parameters

The results of antioxidant parameters in liver and kidney are given in Table 6-7. The data reveal that in diabetic animals, a significant decrease ($p < 0.001$) in SOD and CAT enzyme levels are observed, whereas, the level of TBARS significantly increases ($p < 0.001$) as compared to the normal control rats in liver and kidney. The plant root ethyl acetate extract of the *Asparagus racemosus* will be treated animals, there is a significant ($p < 0.001$) increase in SOD and CAT enzyme levels where as the level of TBARS decreases significantly ($p < 0.001$) compared the diabetic control rats.

1. Effect of extract on the OGTT in HFD and STZ-diabetic rats.

Groups	Treatment	Blood glucose level (mg/dL)				
		0 min	30 min	60 min	120 min	180 min
I	Normal control	89.50 ± 1.02	92.50 ± 1.02	95.83 ± 1.16	96.83 ± 1.01	95.50 ± 0.92
II	Diabetic control	314.33 ± 1.17	324 ± 1.29	344.33 ± 1.11 ^{###}	364.66 ± 0.98 ^{###}	371.66 ± 0.95 ^{###}
III	Glibenclamide (10mg/kg)	311.50 ± 1.08	326.16 ± 1.42	317.50 ± 1.54 ^{***}	327.33 ± 0.84 ^{***}	337 ± 1.12 ^{***}
IV	EEAR(200mg/kg)	312.33 ± 0.91	331.33 ± 0.74	333.66 ± 5.15 ^{***}	344 ± 1.15 ^{***}	346 ± 1.06 ^{***}
V	EEAR(400mg/kg)	328.16 ± 1.16	348.16 ± 1.16	343.16 ± 1.16 ^{***}	346.16 ± 1.16 ^{***}	348.66 ± 1.28 ^{***}

Values are expressed as mean ± SEM (n = 6).

^{###}p<0.001 compared with normal control, ^{***} p<0.001 compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

2. Effect of extract on the blood glucose level in HFD and STZ-diabetic rats.

Groups	Treatment	Blood glucose level (mg/dL)			
		0 day	7 th day	14 th day	21 th day
I	Normal control	89.50 ± 0.84	90.67 ± 0.67	90.83 ± 0.87	90.33 ± 1.05
II	Diabetic control	314.33 ± 1.17	364.33 ± 1.17 ^{###}	394.33 ± 1.20 ^{###}	405.66 ± 1.52 ^{###}
III	Glibenclamide (10mg/kg)	331.50 ± 1.08	248.16 ± 3.41 ^{***}	134.16 ± 1.51 ^{***}	131.83 ± 1.04 ^{***}
IV	EEAR(200mg/kg)	335.33 ± 0.91	265.66 ± 0.99 ^{***}	213.33 ± 1.33 ^{***}	152.16 ± 1.04 ^{***}
V	EEAR(400mg/kg)	320.16 ± 1.16	254.66 ± 1.08 ^{***}	200.16 ± 1.16 ^{***}	150.50 ± 1.31 ^{***}

Values are expressed as mean ± SEM (n = 6).

^{###}p<0.001 compared with normal control, ^{***} p<0.001 compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

3. Effect of extract on body weight, fluid and food intake in HFD and STZ-diabetic rats.

Groups	Treatment	Body weight (g)		Fluid (mL/rat/day)		Food (g/rat/day)	
		Before	After	Before	After	Before	After
I	Normal control	290.83 ± 0.54	339 ± 0.36	144.33 ± 0.61	144.83 ± 0.54	27.67 ± 0.42	28 ± 0.36
II	Diabetic control	284.33 ± 0.61	316.50 ± 0.43 ^{###}	155.50 ± 0.43	175.83 ± 0.60 ^{###}	33.67 ± 0.49	46.16 ± 0.47 ^{###}
III	Glibenclamide (10mg/kg)	267 ± 0.44	314.17 ± 1.49 ^{***}	142 ± 0.57	146.16 ± 0.54 ^{***}	34.83 ± 0.30	38.33 ± 0.30 ^{***}
IV	EEAR(200mg/kg)	276.50 ± 0.50	324.50 ± 0.62 ^{***}	144.50 ± 0.71	149.33 ± 0.42 ^{***}	28.50 ± 0.43	38.66 ± 0.33 ^{***}
V	EEAR(400mg/kg)	283.67 ± 1.13	335.50 ± 0.43 ^{***}	149.83 ± 0.54	155.50 ± 0.43 ^{***}	32.33 ± 0.55	40 ± 0.57 ^{***}

Values are expressed as mean ± SEM (n = 6).

$p < 0.001$ compared with normal control, *** $p < 0.001$ compared with diabetic control.

Two way ANOVA followed by Bonferroni's multiple comparison tests.

4. Effect of extracts on glucose-6-phosphatase and liver glycogen content in HFD and STZ- diabetic rats.

Groups	Treatment	Glucose -6-phosphatase (mmol/min/mg)	Liver glycogen content (mg/g wet tissue)
I	Normal control	0.28 ± 0.04	23.50 ± 0.76
II	Diabetic control	$0.60 \pm 0.05^{###}$	$16 \pm 0.25^{###}$
III	Glibenclamide (10mg/kg)	$0.37 \pm 0.04^{***}$	$21.50 \pm 0.34^{***}$
IV	EEAR(200mg/kg)	$0.34 \pm 0.00^{***}$	$18.67 \pm 0.22^{**}$
V	EEAR(400mg/kg)	$0.39 \pm 0.04^{***}$	$20.33 \pm 0.21^{***}$

Values are expressed as mean \pm SEM (n = 6).

$p < 0.001$ compared with normal control, *** $p < 0.001$ compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

5. Effect of extract on serum lipid and lipoprotein profile in HFD and STZ-diabetic rats.

Groups	Treatment	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I	Normal control	88.17 ± 0.65	79.83 ± 1.04	51.50 ± 1.11	18.50 ± 1.23	15.97 ± 0.20
II	Diabetic control	$174.7 \pm 1.67^{###}$	$187 \pm 1.21^{###}$	$28.17 \pm 0.65^{###}$	$46.17 \pm 0.65^{###}$	$37.40 \pm 0.24^{###}$
III	Glibenclamide (10mg/kg)	$106.3 \pm 1.20^{***}$	$104.3 \pm 0.80^{***}$	$40.83 \pm 0.74^{***}$	$21 \pm 0.36^{***}$	$19.87 \pm 0.16^{***}$
IV	EEAR (200mg/kg)	$119 \pm 0.61^{***}$	$125.3 \pm 1.23^{***}$	$42.17 \pm 0.83^{***}$	$25.50 \pm 0.95^{***}$	$20.07 \pm 0.24^{***}$
V	EEAR (400mg/kg)	$126.2 \pm 0.65^{***}$	$130.3 \pm 0.95^{***}$	$45 \pm 0.81^{***}$	$29.50 \pm 0.80^{***}$	$25.73 \pm 0.24^{***}$

Values are expressed as mean \pm SEM (n = 6).

$p < 0.001$ compared with normal control, *** $p < 0.001$ compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

6. Effect of extracts on antioxidant parameters in HFD and STZ-diabetic rats liver.

Groups	Treatment	CAT (IU/min/mg of tissue)	SOD (IU/min/mg of tissue)	TBARS (nmole of MDA/mg of tissue)
I	Normal control	15.39 ± 0.49	10.70 ± 0.49	11.50 ± 0.42
II	Diabetic control	$6.33 \pm 0.33^{###}$	$4.67 \pm 0.33^{###}$	$24.20 \pm 0.60^{###}$
III	Glibenclamide (10mg/kg)	$10.30 \pm 0.21^{***}$	$8.33 \pm 0.33^{***}$	$14.20 \pm 0.30^{***}$
IV	EEAR (200mg/kg)	$11.80 \pm 0.30^{***}$	$9.08 \pm 0.26^{***}$	$14.70 \pm 0.42^{***}$
V	EEAR (400mg/kg)	$12.20 \pm 0.30^{***}$	$10.08 \pm 0.91^{***}$	$18.70 \pm 0.42^{***}$

Values are expressed as mean \pm SEM (n = 6).

$p < 0.001$ compared with normal control, *** $p < 0.001$ compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

7. Effect of extract on antioxidant parameters in HFD and STZ-diabetic rats kidney.

Groups	Treatment	CAT (IU/min/mg of tissue)	SOD (IU/min/mg of tissue)	TBARS (nmole of MDA/mg of tissue)
I	Normal control	14 ± 0.57	9.50 ± 0.42	10.50 ± 0.56
II	Diabetic control	$5.83 \pm 0.30^{###}$	$4.33 \pm 0.21^{###}$	$22.50 \pm 0.42^{###}$
III	Glibenclamide (10mg/kg)	$10 \pm 0.21^{***}$	$7.17 \pm 0.30^{***}$	$12.20 \pm 0.30^{***}$
IV	EEAR (200mg/kg)	$10.30 \pm 0.36^{***}$	$7.33 \pm 0.28^{***}$	$15.70 \pm 0.49^{***}$
V	EEAR (400mg/kg)	$11.70 \pm 0.33^{***}$	$8.10 \pm 0.28^{***}$	$17.30 \pm 0.21^{***}$

Values are expressed as mean \pm SEM (n = 6).

$p < 0.001$ compared with normal control, *** $p < 0.001$ compared with diabetic control.

One way ANOVA followed by Bonferroni's multiple comparison tests.

We have investigated that the *Asparagus racemosus* Willd which is used in traditional ayurvedic medicine for the treatment of several diseases, has been serving as anti-diabetic potential. Previously, this beneficial and priceless herb was not been investigated for its in-vivo anti-diabetic activity. This herb has clearly recognized the potential of anti-diabetic activity. The treatment goal of diabetes patients is to maintain glycemic levels in control, in both the fasting as well as post-prandial states. Many natural resources have been investigated for the suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine.^[20] The inhibition of their activity, in the digestive tract of humans, is considered to be effective to control diabetes by diminishing the absorption of glucose decomposed from starch by these enzymes.^[21] Therefore, effects have long been sought. In this study we have investigated the anti-diabetic potential of the *Asparagus racemosus* Willd., which is used in traditional ayurvedic medicine for the treatment of several diseases.^[22] This valuable herb was not previously investigated for its in-vivo anti-diabetic activity. However, our study clearly established the anti-diabetic potential of *Asparagus racemosus* Willd, and revealed that the active principles responsible for its potential. Anti-oxidants, may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. Although, in the present study, the enzyme inhibitory activity of these extract were assayed in-vivo, the results from this work should be relevant to the human body. This supportive evidence further increases the medicinal importance of this *Asparagus racemosus* Willd, indicating that this herb is not only beneficial

for diabetes but also may be useful to a number of other human health complications. To maintain glycemic levels in control, in both the fasting and post-prandial states is the treatment goal of diabetes patients. The suppression of glucose production from carbohydrates in the gut or glucose absorption from the intestine have been investigated using natural resources.^[23]

The present study confirms that plant root ethyl acetate extract of the *Asparagus racemosus* has hypoglycemic effect and improved the lipid profile. The efficiency of plant root extract of the *Asparagus racemosus* willd in treating type2 diabetic mellitus was variable and depending on the estimated parameters. Furthermore, plant root extract of the *Asparagus racemosus* willd may be useful as therapy against oxidative stress and liver damage in type 2 diabetic mellitus and is therefore recommended for further studies. These effects should be studied further in human volunteers and diabetic patients.

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