Hypoglycemic, antioxidant and hypolipidemic activity of *Asparagus racemosus* on streptozotocin-induced diabetic in rats

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ABSTRACT

The potential role of the ethanolic extract of *Asparagus racemosus* (Wild) in the treatment of diabetes along with its antioxidant and antihyperlipidemic effects was studied in streptozotocin induced diabetic rats. Oral administration of ethanolic extract of *Asparagus racemosus* (EEAR) 200 and 400 mg/kg/b.w for 21 days significantly decreased the blood glucose level, fluid intake and considerably increased the body weight of diabetic induced rats. EEAR significantly decreased thiobarbituric acid reactive substances and significantly increased in reduced glutathione, superoxide dismutase and catalase in streptozotocin-induced diabetic rats at the end of 21 days of treatment. The study also investigated the antihyperlipidemic potential of EEAR. The results show that the EEAR is promising for development of standardized phytomedicine for the treatment of diabetes mellitus.

Key words: *Asparagus racemosus*, streptozotocin-induced diabetic rats, antihyperglycemic activity, antioxidant potential, antihyperlipidemic effect.

INTRODUCTION

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. Researchers conducted in last few decades on plants mentioned in ancient literature or used traditionally for diabetes have shown anti-diabetic property. There are many plants and their products (active, natural principles and crude extracts) that have been mentioned
or used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity. [1] Among the major phytochemical constituents of plants credited with hypoglycemic action are glycosides, alkaloids, glycans, triterpenes, mucilages, polysaccharides, oils, vitamins, saponins, glycoproteins, peptides, amino acids and proteins. [2]

Over 400 traditional plant treatments for diabetes have been reported, although only a small number of these have received scientific and medical evaluation to assess their efficacy. The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of type 2 diabetes. The World Health Organization Expert Committee on diabetes has recommended that traditional medicinal herbs should be further investigated. [3] Diabetes mellitus (DM) is caused due to deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It is the global problem and number of those affected is increasing day by day. [4]

The abnormalities in lipid metabolism generally lead to elevation in the levels of serum lipids and lipoproteins that turn play an important role in occurrence in premature and severe atherosclerosis which affects patients with diabetes. The most common lipid abnormalities include hypertriglyceridaemia and reduced high density lipoprotein (HDL) cholesterol levels. [5] Diabetes is associated with oxidative stress, leading to an increased production of reactive oxygen species (ROS), including superoxide radical, hydrogen peroxide and hydroxyl radicals or reduction of antioxidant defence system. Implication of oxidative stress in pathogenesis of diabetes is suggested not only by oxygen free radical generation but also due to non enzymatic protein glycosylation, auto oxidation of glucose, impaired antioxidant enzyme and formation of peroxides. Lipid peroxidation is a key marker of oxidative stress results in extensive membrane damage and dysfunction. [6]

Asparagus racemosus constitute of alkaloids, flavonoids, tannins, saponins, phenols, terpenes, polysaccharides and steroids. Asparagus racemosus root extract which contains highest amount of flavonoids, polyphenols and vitamin-C exhibits the greatest antioxidant activity. Asparagus racemosus was used as a bitter, sweet, emollient, cooling, nervinetic, constipating, galactogogue, diuretic, carminative, appetizer, stomachic, antispasmodic and tonic. It is also used in nervous disorders, dyspepsia, diarrhea, dysentery, tumors, inflammation, burning sensation, hyperdipisia, nephropathy, agalactia and general debility. [7-10] But the pharmacological and scientific evidence for its antidiabetic effect is yet to be proved.

**MATERIALS AND METHODS**

**Chemicals:** Streptozotocin from Aldrich and Ascorbic acid, Nitro blue tetrazolium (NBT), sodium nitroprusside, dimethyl sulphoxide, potassium chloride and sodium chloride from Ranbaxy Laboratories Ltd., India. All other chemicals used in the studies were analytical laboratory grades procured from the following manufactures, Loba chemie, ACROS Organics, Merck lab, S.D. Fine chemicals, Fluke.

**Plant Material:** The entire plant of Asparagus racemosus was collected during June 2009, from Nilgiris, Tamilnadu. The plant species was identified and authenticated by Field Botanist, Survey of Medicinal Plants & Collection Unit, Emerald, Niligiris.
Preparation of extract: The collected fresh plant materials were dried in shade (2 days) and then dried in a hot air oven at 25°C for three days and they were made in to coarse powder with the use of mixer grinder. The powder of entire plant of *Asparagus racemosus* obtained were weighed separately and transferred to a round bottomed flask and then went with soxhlet extraction using 95% ethanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried by placing it on a big petriplate on electric water bath (70°C) and then kept in an oven at 30°C for 2 hour. The extract obtained was kept for drying and stored in vacuum desiccators. The percentage yield of the extract was 18.3%.

Animals: Healthy, adult Wistar rats of both sexes (150-220g) were obtained from the central animal house facility J.S.S College of Pharmacy, Ootacamund, Tamilnadu. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3°C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed *ad libitum*, supplied by this institution. All the experiments were performed after obtaining prior approval from IAEC [JSS/IAEC/M.Pharm/Ph.cology/ 06/2009-2010].

Induction of Experimental Diabetes: After 1 week of acclimatization, the rats were subjected to a 16-h fast. Diabetes was induced with a single injection of streptozotocin (STZ) 55 mg/kg body weight i.p. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) [11]. The injection volume was prepared to contain 1.0 ml/kg [12]. After 5 days, blood glucose levels were measured and animals with a concentration of greater than 225 mg/dl were used in the investigation.[13]

Experimental Design: In the experiment, a total of 30 rats (6 normal; 24 STZ diabetic) were used. The rats were divided into five groups of 6 animals each:

- **GROUP I** - Untreated control (0.9% NaCl, 5 ml/kg)
- **GROUP II** - Diabetic control
- **GROUP III** - Diabetic+ Glibenclamide 10 mg/kg b.w i.p
- **GROUP IV** - Diabetic + EEAR 200 mg/kg, p.o
- **GROUP V** - Diabetic + EEAR 400 mg/kg, p.o

The effects of EEAR on STZ-induced diabetic rats were determined by measuring blood glucose levels, liquid intake, and changes in body weight. After 21 d (days) of treatment, all the rats were decapitated after fasting for 16 h.

Biochemical estimation: Fasting blood glucose was measured at different time intervals to check the hyperglycemic state. At the end of 21 days study period blood samples were collected under fasting conditions, the serum obtained after centrifugation was used for the determination of glucose levels. Blood glucose was estimated by glucose oxidase-peroxidase (GOD/POD) method using commercially available diagnostic kits. Serum cholesterol and triglyceride were measured using an Ecoline diagnostic kits.

Determination of *in vivo* Antioxidants activity in liver tissue homogenate

Tissues (liver) were removed and cleared of blood. They were immediately transferred to ice-cold containers containing 0.9% w/v NaCl, homogenized in 0.1 N Tris–HCl buffer (pH 7.4), and
used for the estimation of thiobarbituric acid-reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity.[14-17]

Statistical Analysis: Data a expressed as mean ± S.E.M. for 6 rats in each group. The biochemical parameters were analyzed statistically using one-way ANOVA (Analysis of variance), followed by Tukey’s multiple-comparison test (TMRT). The minimum level of significance was fixed at \( p < 0.05 \).

RESULT

Table 1. summarizes the bodyweight, and liquid intake in normal and experimental animals. There was a significant decrease (\( p < 0.05 \)) in the body weight of the diabetic controls (group II) compared with the normal controls (group I). Administration of EEAR to diabetic rats (groups III, IV) after attaining normal glycemic control increased body weight significantly, comparable to the increase in the body weight of normal rats. Diabetic controls (group II) had a high intake of food and liquids, while the food and liquid intake was decreased in the EEAR -treated groups in comparison to that in the diabetic controls (group II).

Table 2. shows the level of blood glucose in normal and STZ-induced diabetic rats. There was a significant increase in blood glucose level (\( p < 0.05 \)) in diabetic rats when compared with normal controls. Administration of EEAR 200 and 400 mg/kg body weight, and glibenclamide 10 mg/kg significantly decreased blood glucose in diabetic rats (groups III—V).

The effects of EEAR 200 and 400 mg/kg on plasma total cholesterol and triglyceride levels as shown in the Table 2. A significant increase in the cholesterol (\( p < 0.05 \)) and triglyceride (\( p < 0.05 \)) levels were observed in the diabetic group. Treatment with EEAR significantly reduced cholesterol and triglyceride levels in diabetic rats.

The concentrations of TBARS and GSH in tissues in experimental diabetic rats are shown in Table 3. There was a significant elevation of TBARS in STZ-diabetic controls when compared with normal rats. Administration of EEAR and glibenclamide significantly decreased the levels of TBARS in the liver when compared with diabetic controls (group II). There was a significant (\( p < 0.05 \)) decrease in the concentration of GSH in the STZ-diabetic control group when compared with the normal controls. Administration of EEAR 200 and 400 mg/kg body weight reduced the levels of GSH in the liver (\( p < 0.05 \)) during diabetes.

Table:1 Effect of EEAR on Body Weight and Fluid Intake in STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Fluid Intake mL/animal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>Untreated control</td>
<td>194.6±0.13</td>
<td>235 ± 1.46</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>202.7±0.07</td>
<td>168.7 ± 0.07</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (10mg/kg)</td>
<td>206.7±0.07</td>
<td>222.7±0.07a</td>
</tr>
<tr>
<td>Diabetic + EEAR (200 mg/kg)</td>
<td>186.6±0.09</td>
<td>197.7 ± 0.07b</td>
</tr>
<tr>
<td>Diabetic + EEAR (400 mg/kg)</td>
<td>187.7±0.07</td>
<td>200.6±0.11bc</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM (n=6), One-way ANOVA followed by Tukey’s multiple comparison test

\( \text{EEAR} = \text{ethanolic extract of Asparagus racemosus} \)

\( a = \text{compared to untreated control (} P < 0.05 \) \), \( b = \text{compared to diabetic control (} P < 0.05 \) \)

\( c = \text{EEAR (400mg/kg compared to 200 mg/kg) (} P < 0.05 \) \)
### Table 2: Effects of EEAR on Glucose, Cholesterol and Triglyceride Levels in STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose level (mg/dl)</th>
<th>Cholesterol level (mg/dl)</th>
<th>Triglyceride level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>84.0 ± 2.58</td>
<td>57.02 ±1.34</td>
<td>77.20 ± 1.54</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>316.5 ± 1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.9 ±1.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.1 ± 1.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Glibenclamide (10mg/kg)</td>
<td>174.3±1.194&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130.9 ±7.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>143.8 ±2.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EEAR (200 mg/kg)</td>
<td>96.93 ± 1.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102.0 ± 1.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.60±0.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EEAR (400 mg/kg)</td>
<td>87.05c 0.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.68 ± 0.82&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.33±1.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SEM (n=6), One-way ANOVA followed by Tukey’s multiple comparison test

EEAR = Ethanolic extract of Asparagus racemosus

<sup>a</sup>= compared to untreated control (P < 0.05), <sup>b</sup>= compared to diabetic control (P < 0.05)

<sup>c</sup>= EEAR (400mg/kg compared to 200 mg/kg) (P < 0.05)

### Table 3: Effect of EEAR on in-vivo Anti Oxidant Parameter From Liver Homogenate in STZ-Induced Diabetic Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TBARS (µg/mg of protein)</th>
<th>GSH (µg/mg of protein)</th>
<th>SOD (unit/min/gm tissue)</th>
<th>CAT (µmol of H₂O₂/min/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>45.36±0.01</td>
<td>20.67±0.007</td>
<td>3.86 ±0.007</td>
<td>14.96±0.007</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>68.38 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.76 ± 0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.37 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + glibenclamide (10mg/kg)</td>
<td>57.16 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.87 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.57 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.18±0.007&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EEAR (200 mg/kg)</td>
<td>56.7 ±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.15 ±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.26 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.81± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + EEAR (400 mg/kg)</td>
<td>56.75 ±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.27± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.86±0.007&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.07±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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EEAR = Ethanolic extract of Asparagus racemosus

<sup>a</sup>= compared to untreated control (P < 0.05), <sup>b</sup>= compared to diabetic control (P < 0.05)

<sup>c</sup>= EEAR (400mg/kg compared to 200 mg/kg) (P < 0.05)

SOD and CAT levels in experimental animal tissues were summarized in Table 3. There was significant (p <0.05) decrease in SOD and CAT in diabetic-induced groups. Treatment with EEAR of 200 and 400 mg/kg body weight and glibenclamide 10 mg/kg body weight increased the activity of SOD, CAT in the liver to near normal.

### DISCUSSION

Recent studies have clearly demonstrated the importance of medicinal plants in the treatment of experimental diabetes, where oxidative stress induced apoptosis or β -cell death.[18,19]. Oral administration of EEAR showed significant hypoglycemic effects against STZ-induced diabetes in rats. The extract significantly lowered the levels of blood glucose, and TBARS and significantly increased the levels of GSH, SOD and CAT. [20,21]

Generally there is a decrease in the body weight of diabetic untreated animals due to the under utilization of glucose. The final weight of untreated control group was significantly increased than at the beginning of the experiment. The treated groups with EEAR increased the body weight of the animals in a significant manner.[22]
The combination of increased hepatic glucose production and reduced metabolism in peripheral tissues leads to elevated plasma glucose levels.[23]. The treatment with EEAR in STZ induced diabetic rats significantly decreased the elevated serum glucose levels.

Lipid peroxide-mediated tissue damage has been observed in the development of both type I and II diabetes mellitus. It has been observed that insulin secretion is closely associated with lipooxygenase derived peroxides. [24, 25]

The decrease in liver GSH levels represents increased utilization due to oxidative stress [26]. A significant decrease in the levels of GSH was observed in EEAR-treated rats when compared with STZ-induced diabetic controls.

SOD protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (O2 · -), which damages the membrane and biological structures.[27]. CAT has been shown to be responsible for the detoxification of significant amounts of H2O2. [28]. SOD and CAT are the two major scavenging enzymes that remove the toxic free radicals in vivo.

Reduced activities of SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide [29].

Hypercholesterolemia and hypertriglyceridaemia have been reported to occur in streptozotocin diabetic rats [30-33]. The significant increase is observed in our experiment are in accordance with those reports. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyzes triglycerides.

The results of the present investigation of EEAR showed significant antidiabetic activity, antihyperlipidemic and antioxidant properties against STZ induced diabetic rats. Hence, EEAR may be regarded as a promising natural and safe remedy for prevention of diabetic complication.

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