EVALUATION OF ASPARAGUS OFFICINALIS FOR 
ANTI-DIABETIC ACTIVITY IN RATS

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Abstract
Diabetes mellitus is one of the most common endocrine diseases in all populations and all age groups. It is a syndrome of disturbed intermediary metabolism caused by inadequate insulin secretion or impaired insulin action, or both. Insulin is the primary hormone responsible for controlling the uptake, use, and storage of cellular nutrients. The primary goal of therapy is to control symptoms and prevent worsening of neuropathy through improved glycemic control. Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. Asparagus Officinalis have different medicinal properties and is able to treat diabetes and its complications. Diabetes mellitus or hyperglycemia was induced in rats by administration of alloxan monohydrate at dose of 120mg/kg intra peritoneally in normal saline. Statistical comparisons between different groups will be done by using one way analysis of variance. P value <0.05 will be considered as statistically significant. It was found to be in dose dependent way against alloxan induced diabetes in rats. It possess anti diabetic activity and significant effect when compared to alloxan administration. It had shown protection in neuropathy of diabetes and effective peripheral protection.

Keywords: Asparagus Officinalis, Diabetic nephropathy, Anti diabetic activity.

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INTRODUCTION

Diabetes mellitus is one of the most common endocrine diseases in all populations and all age groups. Diabetes mellitus is a chronic metabolic disease caused by inherited and/or acquired deficiency in production and improper functioning of Insulin [1]. The development of diabetes-associated complications is primarily due to the increased glucose concentration and increased polyol pathway activity. In addition, hyperglycemia is involved in most diabetic complications through excessive production of reactive oxygen species [2].

Traditionally, medicinal plants were known to have valuable therapeutic effects, in modern medicine. There is an inverse association between vegetable consumption and chronic disease reduction, such as cancer, CVD and diabetes [3]. Asparagus has been reported to be a rich source of antioxidants, in terms of both quality and quantity [4,5]. In a comparative study among thirty-four fruits and vegetables, asparagus has been placed 7th in the rank of radical scavengers and 13th in ferric-reducing power. The chemical constituents of Asparagus Officinalis have been studied to some extent. The compounds so far reported include saponins [6,7], saccharides [8-10], acetylenic compounds [11] and Sulphur-containing compounds [12-15]. Various reports have suggested that polysaccharides derived from this plant exhibit antioxidant property [16-18].

Fig. 1: Asparagus Officinalis

To our knowledge, there are no studies reporting on any identified compound isolated from A. officinalis as an anti-diabetic. Studies on the extracts of A. officinalis have revealed a wide range of biological activities including diabetes [19]. These activities include anti-tumour, antifungal, diuretic and immunostimulatory effects. Asparagus has also been shown to exert potent...
antioxidant properties. Diabetes mellitus is associated with complications such as nephropathy, retinopathy, neuropathy and cardiovascular disease. This approach can find not only new remedies; but also new lead molecules may be obtained.

Medicinal plants are the main sources of chemical substances with potential therapeutic effects. The use of medicinal plants for the treatment of many diseases is associated with folk medicine from different parts of the world. The Asparagus Officinalis has reported anti-microbial and medicinal properties but the effect of the plant extract on antidiabetic, were not reported yet and so the plant was chosen for the present study. The aim of the present study was to evaluate the anti-diabetic activity of Asparagus Officinalis.

MATERIALS AND METHODS

**Materials**

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium citrate</td>
<td>Virat labs, Hyd, India</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>Finar chemicals limited, Ahmadabad.</td>
</tr>
<tr>
<td>Methanol</td>
<td>E-Merk, Mumbai, India</td>
</tr>
<tr>
<td>Normal saline</td>
<td>Claris life sciences, Ahmadabad, India.</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Finar chemicals limited, Ahmadabad</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Molychem, Mumbai, India</td>
</tr>
<tr>
<td>Alloxan monohydrate</td>
<td>Sigma, St Louis, U.S.A.</td>
</tr>
<tr>
<td>Metformin</td>
<td>MSN Formulations, HYD, India</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>Remi equipment Pvt, Ltd, Hyd, India</td>
</tr>
<tr>
<td>Shimadzu electronic balance</td>
<td>Toshvin Analytical Pvt. Ltd, India</td>
</tr>
<tr>
<td>Shimadzu UV-spectrophotometer</td>
<td>Toshvin Analytical Pvt. Ltd, Mumbai.</td>
</tr>
<tr>
<td>Inverted microscope</td>
<td>Boeckl + co, Hamburg.</td>
</tr>
</tbody>
</table>

**METHODOLOGY**

*Asparagus Officinalis* belongs to the Kingdom Plantae and Order Asparagales and family *Asparagaceae* and its Common name is Garden Asparagus.

**Collection and Authentication of Plant Material**

The Aerial Parts of *Asparagus Officinalis* were collected and authenticated

**Extraction of Plant Material**

The plant is grinded in to a coarse powder with the help of suitable grinder.
Cold Extraction (Methanol Extraction) [20]

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent
The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

Animals
Healthy Adult Male Wister rats of 8-10 weeks old with average weight in the range of 150-180gms were selected. Animals are housed 4 per cage in temperature controlled (27 °C ±3 °C) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water ad libitum. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Induction procedure
Diabetes mellitus or hyperglycemia was induced in rats by administration of alloxan monohydrate at dose of 120mg/kg intra peritoneally in normal saline. After one hour of alloxan administration the animals were given feed ad libitum. The animals were kept fasting overnight and blood glucose levels were estimated before and after 72hrs of alloxan treatment. Animals showing blood glucose levels of >200mg/dl is considered as diabetic and were used for study.

Experimental Study Design for Diabetic screening
Diabetic rats were divided into five groups with each group six animals.

Group-I: Rats served as normal control group.
Group-II: Rats served as diabetic/disease control.
Group-III: Diabetic rats treated with Asparagus Officinalis at a dose  50mg/kg.
Group-IV: Diabetic rats treated with Asparagus Officinalis at a dose of 100mg/kg
Group V: Diabetic rats treated with Metformin (standard drug) at 450mg/kg [21].

The treatment was given for 14 days and blood samples were collected at different intervals.
Collection of blood samples
Blood samples were collected from all the groups of animals at 0, 7, 15th day intervals through puncture of retro orbital plexus and were centrifuged at 3000 rpm for 15 minutes. Serum was separated and stored at -20°C and then used for estimating blood glucose levels.

Experimental Study Design for Diabetic neuropathy screening
Group-I: Rats served as normal control group.
Group-II: Rats served as diabetic/disease control.
Group-III: Diabetic rats treated with Asparagus Officinalis, at a dose 50mg/kg (low dose).

Statistical analysis
All the values will be expressed as mean ± standard deviation (S.D). Statistical comparisons between different groups will be done by using one way analysis of variance. P value <0.05 will be considered as statistically significant.

RESULT AND DISCUSSION

Table 1: Effect of Asparagus Officinalis (EEAO) on serum glucose levels (mg/dL) in diabetic rats

<table>
<thead>
<tr>
<th>Groups / Interval</th>
<th>0th Day</th>
<th>7th Day</th>
<th>15th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>83.3±4.23</td>
<td>79.1±5.36</td>
<td>77.7±5.62</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>283.8±5.01</td>
<td>286.4±12.4</td>
<td>300.3±8.64</td>
</tr>
<tr>
<td>EEAO (50mg/kg)</td>
<td>253.2±20.8</td>
<td>172.2±20.3**</td>
<td>90.3±10.5**</td>
</tr>
<tr>
<td>EEAO (100mg/kg)</td>
<td>230.5±20.4</td>
<td>165.2±16.2***</td>
<td>93.2±10.2***</td>
</tr>
<tr>
<td>Metformin (450mg/kg)</td>
<td>271.0±13.5</td>
<td>80.2±6.4***</td>
<td>70.1±6.3**</td>
</tr>
</tbody>
</table>

All the values are expressed as mean±SD; n=6; ** indicates p<0.01, *** indicates p<0.001 vs. diabetic control.

Fig.1: Effect of EEAO on serum glucose levels (mg/dL) in diabetic rats

All the values are expressed as mean ± SD ; ** indicates p<0.01, *** indicates p<0.001 vs. diabetic control.

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July Issue
Table 2: Diabetic Neuropathy screening by Tail flick response

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean latency period (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>2.18±0.12</td>
</tr>
<tr>
<td>EEAO (50mg/kg)</td>
<td>2.58±0.2</td>
</tr>
<tr>
<td>EEAO (100mg/kg)</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>Diclofenac Sodium (100mg/kg)</td>
<td>2.25±0.35</td>
</tr>
</tbody>
</table>

All the values are expressed as mean±SD ** indicates p<0.01, *** indicates p<0.001 vs. diabetic control.

Fig. 2: Effect of EEAO on Diabetic neuropathy by tail flick in diabetic rats

All the values are expressed as mean±SD; ** indicates p<0.01, *** indicates p<0.001 vs. diabetic control.

Table 3: Diabetic neuropathy screening by Thermal hypoalgesia response

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean latency period(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Normal</td>
<td>3.08±0.4</td>
</tr>
<tr>
<td>EEAO (50mg/kg)</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>EEAO (100mg/kg)</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>Diclofenac Sodium (100mg/kg)</td>
<td>2.70±0.40</td>
</tr>
</tbody>
</table>

Fig. 3: Effect of EEAO on Diabetic neuropathy by Thermal hypoalgesia method in diabetic rats
DISCUSSION
The present study was aimed to evaluate the anti-diabetic activity of *Asparagus Officinalis*. The activity was measured by estimating various biomarkers like blood glucose levels in experimental rats.

In the previous studies, it was shown that alloxan monohydrate induced diabetes mellitus. When given in a dose of 120mg/kg to rats *intra peritoneally* as evidenced in study [22]. In the present study alloxan was administered in a single dose to induce diabetes mellitus in rats at the dose of 120mg/kg. Alloxan forms an increased glucose levels that generates diabetes. Pretreatment with *Asparagus Officinalis* produced significant decrease in glucose levels indicating the protective effect of tissue. On alloxan treatment a dose dependent decrease in glucose levels were observed. Pretreatment *Asparagus Officinalis* and metformin produced significant alteration in glucose levels. Diabetic neuropathy alterations were tested using thermal hypoalgesia and tail flick response [23] and results in comparison to that of the standard drug show that, *Asparagus Officinalis* is neuro protective in diabetic animals.

CONCLUSION
*Asparagus Officinalis* have different medicinal properties and May able to treat diabetes & diabetics complications.

Subjected to acute oral toxicity studies and found that the *Asparagus Officinalis* is safe to use up to the dose of 1000mg/kg.

The *Asparagus Officinalis* was found to be in dose dependent way against alloxan induced diabetes in rats. The reduction of the elevated blood glucose levels in diabetic rats on treatment with the extract at two different concentrations confirmed that methanolic extract of *Asparagus Officinalis* possess antidiabetic activity & has shown significant effect when compared to alloxan administration.

*Asparagus Officinalis* had shown protection in neuropathy of diabetes and effective peripheral protection as shown by results

It needs comprehensive investigations for developing a safe and effective drug. Further research is required to confirm the anti diabetic activity and its complications.

REFERENCES

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