Antidiabetic Potential of *Achyranthes Aspera* Leaves in Rats

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Abstract

Plants have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and inexpensive. In the recent past there has been a tremendous increase in the use of plant based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally. Herbal medicines have a strong traditional or conceptual base and the potential to be useful as drugs in terms of safety and effectiveness leads for treating different diseases. Only preliminary studies have been reported for the antidiabetic activity of the leaves of *Achyranthes aspera*. Therefore, an attempt has been made to evaluate the antidiabetic potential of the ethanol extract of *A. aspera* leaves. It was found that ethanol extract of the leaves of *A. aspera* was active in STZ models in Sprague-Dawley (SD) rats. On further fractionation into four fractions of the ethanol extract, the activity was localized in the chloroform fraction only. Therefore purification of this fraction led to the isolated of four compounds. Out of four compounds two of them showed promising activity.

**Keywords:** Antidiabetic Activity; Achyranthes aspera; STZ models.

Introduction

Plants have been part of our lives since the beginning of time. We get numerous products from plants, most of them not only good and beneficial but also crucial to our existence. Plants are the backbone of all life on earth and an essential resource for human well-being. Everything we eat comes directly or indirectly from plants. Through our history, approximately 7,000 different plant species have been used as food by people. Diabetes is a syndrome characterized by disordered metabolism of carbohydrate, protein and lipid with abnormally high blood sugar resulting from low levels of the hormone insulin with or without abnormal resistance to insulin’s effect [1]. Diabetes mellitus is considered as one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates ten years ago and this may double by the year 2030. India leads the way with its largest number of diabetic subjects in any given country. The prevalence of diabetes mellitus is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels [2].

Traditional medicines most often applies to plants are being employed as adjuvants in the management of diabetes mellitus in many of the Asian countries including India. India has a rich history of using various potent herbs and herbal components for treating diabetes. Medicinal plant or herb have a variety of metabolites, aliphatic compound and aromatic compound, have basic skeleton of organic molecule and have various functional group that makes ability to alter the various metabolic pathway makes them medicinally important. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost. Therefore, investigation on such agents from traditional medicinal plants has become more important.

*Achyranthes aspera* Linn. is an annual, stiff erect herb, about 0.3 to 0.9m high and is a common plant found in wastelands and agricultural fields. The plant is highly esteemed by traditional healers. *A. aspera* is known by different names in India viz. Latjira in Hindi, Apamargah in Sanskrit, and Prickly-chaff flower plant in English [3]. Different parts of the plant have been used as an expectorant, stomach tonic, laxative, anthelmintic, and diuretic, lithotripter, sudorific, demulcent, anti-inflammatory and hematonic in indigenous medicine [4]. Nowadays many studies have been reported diverse actions of *A. aspera* e.g. antiviral [5], antibacterial activity [6, 7], antifertility [8,12], antidiabetic [9,13], positive inotropic effect, spasmylytic to smooth muscle [10], diuretic and purgative [11]. The major aim of the study is to prove the hypoglycemic activity of *A. aspera* leaf extract. The rationale of the present study is to find out the level of efficacy and potency of the leaf extract in accordance with hypoglycemic activity. The leaves of this plant were investigated for its antidiabetic *in vivo* in STZ induced diabetic rat model and to compare the same with metformin, as a standard hypoglycemic drug.

Materials and methods

Plant material

Leaves of *A.aspera* Linn. (2.0 Kg.) were collected from...

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Lucknow, India and was authenticated by Botany Department of the Lucknow University.

**Extraction fractionation and isolation of compounds**

The air dried leaves (2.0 Kg.) were powdered and percolated in 95% ethanol at room temperature for 24 hours, filtered and the process was repeated four times. All the extracts were mixed and filtered. Mixed ethanol extract was concentrated under reduced pressure below 50°C in a rotavapour to a green viscous mass, which was dried under high vacuum for 2 hours to remove the last traces of the solvent. Weight of the dried ethanolic extract 41.5 g. It was used for the screening of antidiabetic activity in STZ models in rats. Further the ethanol extract was fractionated into four fractions, hexane, chloroform, n-butanol soluble and, n-butanol insoluble fractions by maceration with the solvents in increasing order of their polarity (hexane →chloroform → n-butanol). The chloroform extract on chromatography and rechromatography to the isolation of 4 pure compounds. These compounds were characterized by physicochemical techniques (IR, NMR and Mass spectroscopy) and derivatization of suitable derivatives.

**Experimental Animals**

Sprague Dawley rats of either sex, weighing 180-200g were housed in elevated floor mesh cages to prevent coprophagy and were kept in environmentally controlled rooms (temperature 25±2°C and 12 hours light and dark cycle rotation). All experimental protocols were approved by our Institutional Ethical Committee following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy).

**Streptozotocin induced antidiabetic rat model**

Male Sprague Dawley strain albino rats of average body weight 140 ± 20 g having blood glucose profiles between 60 80 mg/dl were selected and were made diabetic by intraperitoneal administration of streptozotocin at a dose level of 45 mg/kg body weight. Streptozotocin (Sigma, USA) was dissolved in 100 mM citrate buffer (pH 4.5) and calculated amount of freshly prepared solution of streptozotocin was injected to overnight fasted rats. Blood glucose profile was again checked after 48 hours by glucostrips (Boehringer Mannheim) and animals showing blood glucose values between 150 to 450 mg/dl were included in the experiments and termed diabetic. The diabetic animals were divided into groups consisting of five animals in each group. Rats of experimental groups were administered suspension of the desired test sample prepared in 1% gum acacia at 100 mg/kg dose level. Animals of control group were given an equal amount of 1% gum acacia. Blood glucose profile of animals of all groups was again checked at 1, 2, 3, 4, 5, 6, 7 and 24 h post administration of test sample. Animals not found diabetic after 48 hours post treatment of the test sample were not considered and omitted from the calculations and termed as non-responders. Food but not water was withheld from the cages during the experimentation. Comparing the AUC of experimental experimental animals as well as by comparing the data with results of standard drugs (Metformin and Glybenclamide) used for diabetic patients and, the control groups determined the percent antihyperglycemic activity. Statistical comparison was made by Dunnett’s test.

**Conclusion**

This paper explains evidence based-information regarding the antidiabetic activity of ethanol extract of *A. aspera* in GLM and STZ models. It was obvious from the result that, the ethanol extract of *A. aspera* played a beneficial role. Further the results shown in table-1 indicated that the chloroform

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**Table 1:** Antihyperglycemic effect of extract/fraction and pure compounds of *A. aspera* in streptozotocin induced diabetic rats.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>% Antihyperglycemic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 h</td>
<td>24 h</td>
</tr>
<tr>
<td>1</td>
<td>95% Aq. Ethanol extract</td>
<td>250</td>
<td>15.1**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>20.6**</td>
</tr>
<tr>
<td>2</td>
<td>Hexane soluble fraction</td>
<td>100</td>
<td>7.61</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform soluble fraction</td>
<td>100</td>
<td>23.14**</td>
</tr>
<tr>
<td>4</td>
<td>n-Butanol soluble fraction</td>
<td>100</td>
<td>10.8</td>
</tr>
<tr>
<td>5</td>
<td>Butanol insoluble fraction</td>
<td>100</td>
<td>4.88</td>
</tr>
<tr>
<td>6</td>
<td>Oleoletic acid</td>
<td>100</td>
<td>16.7**</td>
</tr>
<tr>
<td>7</td>
<td>Ursolic acid</td>
<td>100</td>
<td>12.2*</td>
</tr>
<tr>
<td>8</td>
<td>β-sitosterol</td>
<td>100</td>
<td>8.5</td>
</tr>
<tr>
<td>9</td>
<td>Triacontanol</td>
<td>100</td>
<td>6.2</td>
</tr>
<tr>
<td>10</td>
<td>Metformin (Positive control)</td>
<td>100</td>
<td>26.4 **</td>
</tr>
<tr>
<td></td>
<td>Glybenclamide (Positive control)</td>
<td>100</td>
<td>23.6**</td>
</tr>
</tbody>
</table>

Significance: ***p< 0.001 except marked with asterisk**p<0.01. *p<0.05

Figure 1: *Achyranthes aspera* leaves.
fraction is the active fraction of the ethanol extract. On further purification of this fraction led to the isolation of 5 pure compounds (ursolic acid, oleonolic acid, sitosterol and triaccontane) out of which only ursolic and oleonolic acid showed (Table-1) potent anti-diabetic effect in streptozotocin induced diabetic rats and could therefore be used as a remedy for the treatment of diabetes mellitus.

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References


