ANTI DIABETIC AND ANTHELMINTIC PROPERTIES OF THE LEAVES OF ERYNGIUM FOETIDUM.(L.)

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ABSTRACT

Diabetes is a condition where the amount of glucose in our blood is too high because the body cannot use it properly. This is because our pancreas doesn’t produce any insulin, or not enough insulin, to help glucose enter our body’s cells or the insulin that is produced does not work properly. Helminthiasis is infestation with one or more intestinal parasitic worms or hookworms. In the present study anthelmintic and anti-diabetic potentials of the methanolic extracts of the leaves were evaluated and the study reveals that the leaves of Eryngium foetidum possesses significant anthelmintic and anti-diabetic properties.

KEYWORDS: Eryngium foetidum, Anti-diabetic activity, Anthelmintic activity, alpha glucosidases, Piperazine citrate

INTRODUCTION

Eryngium foetidum L. is a biennial herb which is used extensively as a medicinal plant in most tropical regions. It is of increasing importance as a spice plant cultivated in India. It also possesses a wide range of ethno medicinal uses including treatment for burns, earache, fevers, hypertension, constipation, fits, asthma, stomach ache, worms, infertility complications, snake bites, diarrhoea and malaria. Chemical evaluation of the leaves indicated the presence of flavonoids, tannins, a saponin and several triterpenoids. Across the world, one person in 12 now lives with diabetes. Type 2 diabetes is by far the most common type and the good news is that up to 80% of type 2 diabetes is preventable by changing diet, increasing physical activity and improving the living environment. Treatments aim to keep...
the level of blood glucose as normal as possible. For type 1 diabetes, involves regular injections of insulin, a regulated diet and the careful monitoring of blood glucose levels. As people with type 2 diabetes still produce some insulin, diet alone is often effective, although oral anti-diabetic drugs and insulin injections may also be required. Many common herbs and spices are claimed to have blood sugar lowering properties that make them useful for people with or at high risk of type 2 diabetes.

A number of clinical studies have been carried out in recent years that show potential links between herbal therapies and improved blood glucose control, which has led to an increase in people with diabetes using these more natural ingredients to help manage their condition. As per WHO, only few drugs are frequently used in the treatment of the parasite infections. However, increasing problems of development of resistance in helminthes against anthelmintic drugs have led to the proposal of screening medicinal plants for their anthelmintic activity. Plants provide a huge part of traditional veterinary practices and are a rich source of herbal anthelmintics of veterinary importance for centuries. There is an increase in interest of ethnomedical practices across the world for the use of medicinal plants in treatment of helmintic diseases.

MATERIALS AND METHODS
PLANT MATERIAL
The Fresh leaves of *Eryngium foetidum* were collected from the Cheriyoor, Kuttiyeri Village of Kannur District of Kerala state. The plant material was identified and a voucher specimen was deposited in the Department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kerala.

PREPARATION OF THE EXTRACT
The leaves were shade dried and powdered mechanically and sieved and stored in an air tight container. The extraction was carried out by hot percolation method using Soxhlet apparatus. The solvent used was methanol. The extract was concentrated to dryness under controlled temperature. The extract was preserved in refrigerator till further use. A preliminary phytochemical screening has also been carried out for the detection of various chemical constituents.

INHIBITION OF ALPHA GLUCOSIDASES ENZYME
*Invitro* method employed in anti-diabetic studies as per Hamdan II *et.al.*, 2004. The inhibitory activity was determined by incubating a solution of starch substrate (2 % v/v
maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method.  

CALCULATION OF 50% INHIBITORY CONCENTRATION (IC₅₀)  
The concentration of the plant extracts required to scavenge 50% of the radicals (IC₅₀) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by:

\[
I \% = \frac{(Ac-As)}{Ac} \times 100
\]

* Ac is the absorbance of the control.
* As is the absorbance of the sample.

ANTHELMINTIC STUDIES  
Indian earthworm *Pheretima posthuma* were collected from the water logged areas of soil in Koratagere Village of Tumkur District of Karnataka. *Tubifex tubifex* were collected from Aquarium of the local market at Kannur, Kerala. The parasites collected were confirmed before the study. The average size of *Pheretima posthuma* were 5-8.5 cm and that of *Tubifex tubifex* were 2.0 cm. They were washed with water to remove the unwanted materials. All parasites were washed with saline before the evaluation. The anthelmintic assay was carried out as per the method of Ajayieoba E. O. et al. with minor modifications. Before starting the study, all the test solutions and standard drug solutions were prepared freshly. Six groups of earth worms of approximately equal size were released in to 25 ml solutions of three different concentrations (50,100,250mg/ml) in Petri dishes containing above solutions of extracts. Piperazine citrate was used as reference standard and saline as control. Determination of time of paralysis and time of death of the worms were done. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water with a temperature not exceeding 50°C followed by observable colour changes in their body.
RESULTS
EVALUATION OF ANTIDIABETIC ACTIVITY

The methanolic extract of the leaves revealed a significant inhibitory action on α-glucosidase enzyme. The percentage inhibition at 250-1500 μg/ml concentrations of the extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 69.77 ± 0.8439 to 40.31 ± 0.2436 for highest concentration to the lowest concentration of 250 μg/ml. The concentration required for 50% inhibition (IC$_{50}$) was found to be 611.49 ± 4.29

Table 1 α-glucosidase inhibition by leaves of *Eryngium foetidum* methanol extract

<table>
<thead>
<tr>
<th>Sl. NO</th>
<th>Concentration (Micro grams/ml)</th>
<th>Percentage Inhibition</th>
<th>IC$_{50}$ (Micro grams/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>40.31 ± 0.2436</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>47.44 ± 0.6978</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td>53.39 ± 0.5980</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>62.99 ± 0.7320</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1500</td>
<td>69.77 ± 0.8439</td>
<td>611.49 ± 4.29</td>
</tr>
</tbody>
</table>

(Experiments were carried out in triplicate and values are expressed as the mean ± SEM)

EVALUATION OF ANTHELMINTIC ACTIVITY

The experiments were done on adult Indian earthworm *Pheretima posthuma* and also in the aquarium worm, *Tubifex tubifex*, because they belong to same group of Annelida (Mueller, 1774). An anthelmintic drug can act by causing paralysis of the worm or by damaging its cuticle, leading to partial digestion or rejection by immune mechanisms. Anthelmintic drugs can also interfere with the metabolism of the worm and since the metabolic requirements of these parasites vary greatly from one species to other drugs that are highly effective against one type of worm can be ineffective against another.

Table 2 Anthelmintic Activity of the methanolic extract of *Eryngium foetidum*

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Conc.(mg/ml)</th>
<th><em>Pheretima posthuma</em></th>
<th><em>Tubifex tubifex</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time for Paralysis in minutes</td>
<td>Time for Death in minutes</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>50</td>
<td>84±0.045</td>
<td>145±0.069</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>54±0.034</td>
<td>59±0.049</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>40±0.057</td>
<td>51±0.025</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>50</td>
<td>155±0.011</td>
<td>217±0.039</td>
</tr>
<tr>
<td>(Standard)</td>
<td>100</td>
<td>129±0.011</td>
<td>197±0.037</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>110±0.079</td>
<td>158±0.034</td>
</tr>
</tbody>
</table>

Saline was used as control
(Values are mean ± S.E.M. from six observations.)
**DISCUSSIONS AND CONCLUSION**

The intestinal enzymes α-glucosidase is found to be very important in carbohydrate digestion and glucose absorption. The suppression of the activity of such digestive enzyme would delay the degradation of starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation. Inhibition α-glucosidases reduced the high postprandial blood glucose peaks in diabetes. The study reveals that methanolic extract of the leaves efficiently inhibits alpha glucosidase enzymes in vitro in a dose dependent manner. The anti-diabetic action of the plant can also be attributed to the intestinal alpha glucosidase inhibitory activity, so further the compound isolation, purification and characterization which are responsible for inhibiting activity, has to be evaluated for the usage of the leaves of *Eryngium foetidum* as an anti-diabetic agent.

The route and dose of anthelmintic drugs are important and must be chosen carefully since parasitic worms cannot be relied upon to consume sufficient amount of drug to be effective. The detailed literature survey of the plant reveals its usage among the tribals in treating intestinal worm infections. The data obtained in the study could provide a rational for the traditional use of this plant as an anthelmintic.

The extracts of the plant displays a significant anthelmintic activity in dose dependent manner. The anthelmintic activities of methanolic extract of the leaves were comparable with that of standard drug. Piperazine citrate is effective in a broad range of helminth infections. The mechanism of action involves inhibition of the glucose uptake system leading to a lethal depletion of energy reserves in the helminthes. Phytochemical screening of the leaves reveals the presence of polyphenolic compound as one of the major chemical constituents. The probable mechanism of action of the leaves of *Eryngium foetidum* (L.) as an anthelmintic could be due to the presence of high poly phenolic content accumulated in the leaves. In addition the phytochemical screening of the leaf extract also showed that the plant contain a number of terpenoids. Terpenoids in plants are also responsible for anthelmintic activity. This study strongly supports the traditional use of the plant as an anthelmintic agent.

**REFERENCES**