ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF ISOLATED STIGMASTEROL FROM METHANOL EXTRACT OF ACALYPHA INDICA

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ABSTRACT

Isolated stigmasterol from methanolic solvent extract of acalypha indica were tested for antidiabetic activity and antioxidant activity using streptozocin induced diabetic rats compared with standard and DPPH method for antioxidant activity. The results expressed that stigma sterol has significant protection and maximum reduction in blood glucose was observed in streptozocin induced diabetic rats. in DPPH method maximum antioxidant showing the freeradical scavenging activity of DPPH .the result of this comprehensive study reveal that Isolated stigmasterol acalypha indica methanol extract shown stastically significant antidiabetic activity comparison to the standard glibenclamide.

KEYWORDS: Acalypha indica, antidibetic activity, streptozocin induced method, Antioxidant acivity, DPPH method.

INTRODUCTION

Acalypha indica in telugu name murkonda or kuppichettu. its botanical name acalypha indica, family Euphorbiaceae, plant uses, antibacterial,[1] antibacterial,[2] antivatha,[3] anthelmintic,[4] expectorant,[5] Diuretic,[6] Emetic,[7] Acalypha indica in English Indian acalypha. The leaf juice of leaves is boild along with gingelly and this is applied externally over pain ful areas of body. The leaves are ground with salt and externally ove skin infection like scabies. The leaves are ground along with manjal and applied externally over ulcers, poisonous, bites. The paste leaves along with lime or chunambu is externally applied over painful arthritis. The leaf juice is externally applied over head ache. The dried leaf powder is bandaged over the bed sore areas and hence produce an anthelmintic action. The decoction of roots is also used to induce purgation.[8,9]
MATERIALS AND METHODS

Plant materials were washed with running tap water and dried under shade conditions then it is subjected to soxhlet extraction method after extraction again it is treated for chemical isolation methanolic extract of acalypha indica subjected to column chromatography method. In which different solvent ratio is used to collect the fractions from column chromatography which is chloroform: ethanol (9.5:0.5) is used for isolation of stigmasterol.

Streptozotocin induced diabetes

Adult inbred wistar albino rats (35 numbers) of either sex were over night fasted and received a freshly prepared solution of streptozotocin (STZ), [Sigma Chemical Co, St Louis, MO, USA], (50mg/kg) in 0.1 M citrate buffer, pH 4.5, injected intraperitonially. After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation. Out of 35 animals subjected for diabetes induction, 6 animals died before grouping and five animals were omitted from the study, because of sub diabetic condition (118mg/dl) and (122mg/dl). The remaining 24 animals 4 groups of 6 animals were formed and used for the experimentation. In the present study, glibenclamide (4 mg/kg body weight) was used as the standard drug.

Grouping of animals

Group I : served as normal control
Group II : served as diabetic control and received STZ
Group III : stigmasterol 5mg
Group IV : stigmasterol 10mg
Group V : Diabetic rats treated with and glibenclamide 4 mg/kg/p.o.

Served as Standard

Fasting blood glucose estimation was done at 0, 2, 4 and 6 hr after the treatment. Drug treatment was continued for 21 consecutive days. The fasting blood glucose levels were estimated on days 0, 1, 7, 14, and 21.
INVITRO ANTIOXIDANT ACTIVITY

DPHP scavenging activity of stigmasterol

1,1- diphenyl-2-picrylhydrazyl (DPPH, 0.004%) solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol and kept it overnight in dark place for the generation of DPPH radical. An aliquot of 3 ml of 0.004% DPPH solution in ethanol and 0.1 ml of test sample at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of isolated e stigmasterol. The percentage inhibition of free radical by the test sample was calculated using the formula:

\[
\text{DPPH scavenging effect (\%) = } \frac{(A_0 - A_1)}{A_0} \times 100
\]

Inhibitory ratio =Where, \(A_0\) is the absorbance of control;
\(A_1\) is the absorbance with addition of test sample.

RESULT AND DISCUSSION

In diabetes acivity is dose dependent of stigmasterol 5mg ,10mg compared with the standard solution. control is normal and stigmasterol 5mg solution was exhibit the inhibition of diabetic activity is there. Again stigmasterol 10 mg sample was inhibition activity is there when compare to the standard solution. where as incase of antioxidant activity based on increased concentration produce the antioxidant activity.

Table-1: Antidiabetic activity of isolated stigmasterol.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (Kg per body weight)</th>
<th>Blood Glucose(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>I</td>
<td>Control(5ml)</td>
<td>5 ml cmc</td>
<td>92.1±16</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control (STZ500mg)</td>
<td>5ml</td>
<td>253.6±36</td>
</tr>
<tr>
<td>III</td>
<td>Test 5mg (stigmasterol)</td>
<td>5 mg</td>
<td>240.5±54</td>
</tr>
<tr>
<td>IV</td>
<td>Test 10 (stigmasterol)</td>
<td>10mg</td>
<td>246.0±35</td>
</tr>
<tr>
<td>V</td>
<td>Standard(4mg glibenclamide)</td>
<td>4 mg</td>
<td>243.3±45</td>
</tr>
</tbody>
</table>
Table-2: Antioxidant activity of isolated stigmasterol.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Inhibition of ascorbic acid</th>
<th>% inhibition of stigmasterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ugi/ml</td>
<td>22.8±0.2</td>
<td>16.7±0.3</td>
</tr>
<tr>
<td>10 ugi/ml</td>
<td>39.8±0.1</td>
<td>34.2±0.2</td>
</tr>
<tr>
<td>25 ugi/ml</td>
<td>53.6±0.3</td>
<td>47.20±03</td>
</tr>
<tr>
<td>50 ugi/ml</td>
<td>59.6±0.2</td>
<td>51±0.5</td>
</tr>
<tr>
<td>100 ugi/ml</td>
<td>64.6±0.6</td>
<td>56.7±0.3</td>
</tr>
<tr>
<td>250 ugi/ml</td>
<td>73.1±0.1</td>
<td>64.7±0.1</td>
</tr>
<tr>
<td>500 ugi/ml</td>
<td>78.9±0.2</td>
<td>66±0.2</td>
</tr>
</tbody>
</table>

REFERENCES
5. Rangari V. Pharmacognosy and Phytochemistry-II; Traditional drugs of India. Ist ed. career publication, 2008; 177-178.