INTRODUCTION

Diabetes mellitus consists of a group of syndrome characterized by hyperglycemia, altered metabolism of lipids, carbohydrates, and proteins; resulting from defects in insulin secretion, its action, or both. It is a chronic metabolic disorder which affects a significant population worldwide. The total number of people globally affected by diabetes was reported to be 171 million in 2000 and is projected to be 366 million by 2030 with a prevalence rate of 4.4 percent. Greatest number of cases being expected in China and India.

It is a major cause of morbidity and mortality. Present drugs which are used for the treatment of this disease are mainly insulin, sulphonylureas and biguanides. All these drugs are associated with adverse effect and not able to control metabolism adequately. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. There is growing interest in herbal remedies due to these reasons.

From ancient time plant species are used in the treatment of various diseases. It is an important ingredient in traditional medicine. But yet only about five percent of the total plant species has been thoroughly tested for its safety and efficacy.

H. antidysenterica commonly known as kurci, kurchi or kutaj is one of the important medicinal herbs of the family Apocynaceae. Its seed is known as Indrayava or indrakaj and efficacy of ethanolic extract of its seed in diabetic rats is still a challenge. There are few studies of different parts of this plant related to its hypoglycemic effect. There are studies of different parts of this plant related to its hypoglycemic effect. Fifty percent ethanolic extract of fruit has shown hypoglycemic activity in rats. Aqueous and alcoholic extract of its bark has reversed the hyperglycemic condition produced by streptozotocin injection. Scientific data related to the antidiabetic efficacy of ethanolic extract of its seed in diabetic rats is still a challenge.

Recent study reflects the use of these traditional medicinal plants against malaria, multiple antibiotic resistant enteropathogenic E. coli and in gut motility disorder. There are few studies of different parts of this plant related to its hypoglycemic effect. Fifty percent ethanolic extract of fruit has shown hypoglycemic activity in rats. Aqueous and alcoholic extract of its bark has reversed the hyperglycemic condition produced by streptozotocin injection. Scientific data related to the antidiabetic efficacy of ethanolic extract of its seed in diabetic rats is still a challenge.

The purpose of this study was to investigate the efficacy of ethanolic extract of H. antidysenterica seeds in STZ – induced diabetic rats and evaluating its effect in blood cholesterol, total protein, alkaline phosphatase, aspartate transaminase, alanine transaminase, urea, creatinine and uric acid.

MATERIALS AND METHODS

H. antidysenterica seed were collected from the local market of Ranchi. Voucher specimens were deposited in the department of botany, Ranchi College Ranchi, of Ranchi University (voucher no. RIMS/15111/01). Taxonomist of this Department confirmed its identity.

Plant extract preparation

The seed were washed and dried in the shade. It was powdered in the grinder. Ethanolic extract (with 90% ethanol) of it were concentrated in evaporator under reduced presser at 40°C. The extract was stored at 0-4°C.
Experimental animals

Permission for the study was taken from institutional animal ethics committee. Healthy albino rats of either sex of 2-2½ months old of body weight 125-150g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 hours. It was acclimatized for seven days. All animals were given standard rat feed and water ad libitum.

Drugs and chemicals used

Glibenclamide, streptozotocin and sodium citrate buffer were used in this study. The chemicals used in this study were of laboratory grade.

Acute toxicity test

Acute oral toxicity for the ethanolic extract of H. antidysenterica seed (EHA) was carried out on the basis of OECD guideline 425. The dose of the drug was administered by calculating the weight; as 3000 mg/kg body weight. At first a single animal was tested. It was observed carefully to watch mortality and toxicity for 24 hours. Thereafter four other animals were also given EHA according to their body weight subsequently. The animals were marked and housed individually in cages. Animal were watched for 14 days. No animal died in this period. Therefore, the LD50 is greater than 3000 mg/kg.  

Oral glucose tolerance test

Oral glucose tolerance test was done in overnight (18 hour) fasted rats. Rats were divided into four groups (N=6) and distilled water (10 ml/kg), EHA (300 and 600mg/kg b. w.), glibenclamide (5mg/kg) were administered with glucose (2g/kg b.w.). Blood was drawn from the tip of the tail at 0, ½, 1 and 2 hr. interval and blood glucose level was estimated with the help of glucometer using strip method (ascensia entrust,bayer pharma).

Antidiabetic study

Diabetes was induced in overnight fasted rat by single intraperitoneal injection of freshly prepared streptozotocin (Sisco research laboratory) dissolved in 0.1 M ice cold sodium citrate buffer of pH 4.4 at a dose of 40 mg/kg b.w. Ten percent glucose solution were allowed to overcome the drug– induced hypoglycemia. 3rd day plasma glucose level of each rat was determined for confirmation of diabetes. Rats with plasma glucose level above 300 mg/dl were considered a diabetic and used subsequently for further study.

Rats were divided into four groups, each group having six animals. Group 1 served as diabetic control and received distilled water 10 ml/kg b.w. Group 2nd and 3rd received ethanolic extract of H. antidysenterica at a dose of 300mg/kg and 600mg/kg b.w, p.o respectively. Group 4 received the standard drug glibenclamide 5mg/kg p.o. Blood glucose were estimated before starting the treatment and weekly up to the 4th week. Blood cholesterol, triglyceride, aspartate transaminase, alanine transaminase, alkaline phosphatase, total protein, urea, creatinine and uric acid were determined at the end of the experiment.

The drug was given once daily after determining hyperglycemia. Blood was drawn in fasting condition from the tip of the tail and blood glucose level was estimated weekly till four week with the help of glucometer using strip method. At the end of the experiment (at four week) blood sample were taken by retro orbital sinus under ether anesthesia by bleeding method for measuring various biochemical parameter mentioned as above.

STATISTICAL ANALYSIS

Results were expressed as mean with standard deviation. The data were analyzed using analysis of variance (ANOVA). Group means were compared by Post Hoc test. Values were considered statistically significant with p ≤ 0.05.

RESULTS

Acute toxicity studies

Acute toxicity studies revealed the nontoxic nature of the ethanolic extract of H. antidysenterica. It did not produce changes in behavior, and general activity of the animal at 3000 mg/kg dose. All the animals were alive, healthy and active during the observation period. No death was observed at this dose. The fact that the LD50 of ethanolic extract of H. antidysenterica is above 3000 mg/kg is an indication that the extract could be considered relatively safe, when given orally.

Oral glucose tolerance test

EHA significantly reduced plasma glucose levels ½ hr after administration of glucose in euglycemic rats (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>0 hr. (mg/dl)</th>
<th>½ hr. (mg/dl)</th>
<th>1 hr. (mg/dl)</th>
<th>2 hr. (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (Distilled water 10ml/kg)</td>
<td>76.5±2.51</td>
<td>92±2.83</td>
<td>103±3.34</td>
<td>87±0.89</td>
</tr>
<tr>
<td>EHA (300mg/kg)</td>
<td>78±3.22</td>
<td>88.67±2.66</td>
<td>96.3±3.83</td>
<td>79.67±3.5</td>
</tr>
<tr>
<td>EHA (600mg/kg)</td>
<td>80.16±2.99</td>
<td>90.33±3.01</td>
<td>99.17±2.04</td>
<td>80±2.13</td>
</tr>
<tr>
<td>GLB (5mg/kg)</td>
<td>81.33±2.34</td>
<td>89.4±2.2</td>
<td>93.8±3.05</td>
<td>74.1±4.1</td>
</tr>
</tbody>
</table>

Values are mean ± SD of 6 rats in each group. *P < 0.05 when compared with vehicle-treated group (Post hoc test).

Changes in Body Weight

Diabetic rats showed a decrease in body weight during the experimental period. 21.94% decrease was noted at the end of 4th week. H. antidysenterica and glibenclamide treated diabetic group rat showed significant increase in weight. Percentage increase in body weight in these (H. antidysenterica 300 and 600mg/kg b.w and glibenclamide) treated group were respectively 24.75, 24.66 and 27.51 in four weeks (Table 2).
TABLE 2: Effect of ethanolic extract of H. Antidysenterica seed on body weight of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>0 Week</th>
<th>1 Week</th>
<th>2 Week</th>
<th>3 Week</th>
<th>4 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC</td>
<td>132.17±7.03</td>
<td>127±7.03</td>
<td>120.83±6.98</td>
<td>111.83±5.83</td>
<td>103.17±3.97</td>
</tr>
<tr>
<td>EHA1</td>
<td>136.67±5.20</td>
<td>143.67±6.65</td>
<td>151.5±5.47</td>
<td>159.5±3.73</td>
<td>170.5±2.51</td>
</tr>
<tr>
<td>EHA2</td>
<td>133.67±7.08</td>
<td>143.67±6.65</td>
<td>153±6.20</td>
<td>161.33±5.57</td>
<td>166.83±3.82</td>
</tr>
<tr>
<td>GLB</td>
<td>131.5±7.04</td>
<td>138.5±7.42</td>
<td>146.83±7.76</td>
<td>153.5±10.25</td>
<td>167.67±7.23</td>
</tr>
</tbody>
</table>

Mean body weight (g) of diabetic control (DC), ethanolic extract of H. antidysenterica seed 300 and 600mg/kg (EHA1 & EHA2) and glibenclamide 5mg/kg (GLB) treated group albino rats during the experimental period. Values are mean ±SD of 6 rats in each group. *P<0.001 when compared with diabetic-control group (Post hoc test).

Blood glucose level

After streptozotocin injection all the rats developed diabetes and blood glucose level was at about 330 mg/dl. Significant reduction (P < 0.05) in blood glucose levels was observed weekly in EHA treated diabetic rats (in a dose of 300 and 600mg/kg) (Table 3). The decrease in blood glucose level in EHA treated group was less in comparison to glibenclamide treated rats.

Table 3: Effect of ethanolic extract of H. antidysenterica seeds on STZ induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>0 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Control</td>
<td>329.5±13.46</td>
<td>334.3±6.62</td>
<td>327.33±5.28</td>
<td>327.5±10.82</td>
<td>334.5±7.09</td>
</tr>
<tr>
<td>EHA1 treated</td>
<td>329.33±8.76</td>
<td>210.5±28.82</td>
<td>179±10.20</td>
<td>152.67±10.5</td>
<td>133.67±6.71</td>
</tr>
<tr>
<td>EHA2 treated</td>
<td>328.33±8.74</td>
<td>244.8±11.2</td>
<td>171±9.36</td>
<td>139.5±20.96</td>
<td>133±3.35</td>
</tr>
<tr>
<td>GLB treated</td>
<td>332.83±6.01</td>
<td>231.17±8.99</td>
<td>163.83±16.15</td>
<td>119.67±11.81</td>
<td>88.67±2.16</td>
</tr>
</tbody>
</table>

Mean fasting blood glucose level (mg/dl) of diabetic control, ethanolic extract 300 and 600mg/kg (EHA1 & EHA2) and glibenclamide 5mg/kg (GLB) treated group albino rats during the experimental period. Values are mean ± SD, n=6 in each group. *P < 0.001 when compared with diabetic control rats (Post hoc test).

Effect on biochemical parameters

Administration of Ethanolic extract of H. antidysenterica in 300, and 600 mg/kg dose for 4 weeks significantly decreased the total cholesterol (P < 0.001) and triglyceride (p < 0.001) level in albino rats in comparison to diabetic control rats [Table 4].

A significant decrease in AST (P < 0.05), ALT (P < 0.001), urea (P < 0.05), and serum creatinine(P < 0.05) level was found in EHA (in both 300 and 600mg/kg dose) treated diabetic rats. Total plasma protein was significantly maintained in EHA treated rat.

Table 4: The effect of the ethanolic extract of H. antidysenterica seed on biomedical parameters

<table>
<thead>
<tr>
<th></th>
<th>DC</th>
<th>EHA1</th>
<th>EHA2</th>
<th>GLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>140±3.41</td>
<td>84±1.41*</td>
<td>82±2*</td>
<td>87±3.74</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>182.5±4.23</td>
<td>140±6.23*</td>
<td>136.8±5.67*</td>
<td>100±2.53</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>65.33±3.78</td>
<td>45±2.28*</td>
<td>46±2.9</td>
<td>43±2.37</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>49±5.44</td>
<td>43±2.1*</td>
<td>47±3.46</td>
<td>39±2</td>
</tr>
<tr>
<td>ALK (U/L)</td>
<td>188.5±5.13</td>
<td>167.8±4.9*</td>
<td>172.50±4.2*</td>
<td>166±3.95</td>
</tr>
<tr>
<td>Total protein (mg/dl)</td>
<td>4.4±0.28</td>
<td>7.90±0.01*</td>
<td>8.2±0.05*</td>
<td>7.83±0.02</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>0.48±0.02</td>
<td>0.55±0.05</td>
<td>0.38±0.08*</td>
<td>0.32±0.12</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>2.91±0.16</td>
<td>2.32±0.29</td>
<td>2.48±0.19*</td>
<td>2.28±0.29</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.48±0.19</td>
<td>2.55±0.15</td>
<td>2.32±0.29</td>
<td>2.28±0.29</td>
</tr>
</tbody>
</table>

The effect of the ethanolic extract of H. antidysenterica seed on biochemical parameters of diabetic control (DC), ethanolic extract EHA 300 and 600mg/kg (EHA1 & EHA2) and glibenclamide 5mg/kg (GLB) treated group albino rats during the experimental period. Data are expressed as mean ± SD, (N=6). *P < 0.001, †P < 0.05 when compared to diabetic control rats.

DISCUSSION

In glucose tolerance test a significant reduction in plasma glucose levels was found in EHA treated euglycemic rat when it was compared to normal control rats. It is probably due to its antihyperglycemic effect by retarding the carbohydrate absorption from intestine through the inhibition in α-glucosidase activity. 12

This study shows the efficacy of EHA seed in streptozotocin induced diabetic rats. Semiliar results of the methanolic extract of this plant seeds were reported by mana et al.13 STZ caused the destruction of pancreatic β cells in albino rats. Consequences to it animals developed marked hyperglycemia. Over production of glucose and decreased utilization by the tissues is the fundamental basis of hyperglycemia in diabetes mellitus. The possible hypoglycemic activity by EHA may be through potentiating action on β cell of islets or stimulation of blood glucose uptake by peripheral tissue or/and inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles. 14

Decrease in body weight in streptozotocin induced diabetic rats and weight gain in EHA treated group (table 2) is due to loss of tissue protein15 and muscle wasting in the former and having beneficial effect in preventing loss of body weight and in catabolic process in EHA treated groups.
Significant increase in weight in EHA treated group of albino rats in comparison to vehicle treated diabetic rats indicating that ethanolic extract of H. antidysenterica had beneficial effect in preventing loss of body weight of diabetic rats.

Insulin deficiency leads to various metabolic abnormalities in animals. Increased ALT, AST, alkaline phosphates, serum cholesterol, serum triglyceride and decreased total protein are indication of these abnormalities. Restoration of the level of biochemical enzymes in H. antidysenterica and glibenclamide treated albino rats are indication of better control of blood sugar level in this group.

REFERENCES


CONCLUSION

The ethanolic extract of H. antidysenterica seed has antihyperglycemic activity as it lowers serum glucose level in diabetic albino rats and significantly increases glucose tolerance. It prevents weight loss in diabetic rats and corrects altered biochemical parameters e.g. serum cholesterol, triglyceride, aspartate transaminase, alanine transaminase, alkaline transferase, total protein, urea, creatinine and uric acid to near normal physiological range. This is an indication of its better metabolic control and potent antidiabetic property. Hence, further studies are required to know the exact mechanism of action and compound responsible for antidiabetic effect.