Antidiabetic Activity of Few Indian Medicinal Plants Vs their Combination in Alloxan Induced Diabetic Rats

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ABSTRACT
In the present study we have compared the antidiabetic activity of extracts of karela fruit, kutki rhizome, neem leaf, tulsi leaf and their combination in alloxan induced diabetic rats at two dose levels, 200 mg/kg and 400 mg/kg respectively. All these extracts and their combination significantly reduced the blood glucose level and exhibited marked antidiabetic activity. The combination of karela : kutki : neem : tulsi (2:1:1:1) showed the most effective activity near similar to the standard drug, Glibenclamide, 4 mg/kg. Treatment with these extracts significantly reduced the elevated biochemical parameters such as serum urea, creatinine, cholesterol and triglyceride in alloxan induced diabetic rats indicating its use in diabetic complications. This study would be helpful in development of an antidiabetic herbal formulation.

Keywords: Diabetes mellitus, alloxan, glibenclamide, Momordica charantia, Picrorrhiza kurroa, Azadirachta indica, Ocimum sanctum.

INTRODUCTION

Diabetes mellitus (DM) is the name given to a group of disorders characterized by chronic hyperglycaemia, polyurea, polydipsia, polyphagia, emaciation and weakness due to disturbance in carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or insulin action (1). It is a global disease that is a major cause of morbidity in the world. The worldwide prevalence of diabetes mellitus is expected to be more than 240 million by the year 2010 (2).

Commonly practiced pharmacologic treatment of diabetes mellitus includes oral hypoglycaemic agents and insulin. There is an increasing demand by patients for the use of natural products and other dietary modulators with antidiabetic activity. This tendency is because insulin, to date, cannot be used orally and its repeated injections have many undesirable adverse effects. In addition, certain oral hypoglycaemic agents are not effective in lowering the blood sugar in chronic diabetic patients. The global information on ethnobotanicals includes about 800 medicinal plants are used for controlling diabetes mellitus. A number of plants, including vegetables, are commonly consumed in India and other parts of the world; and many of these are purported to possess antidiabetic potential (1). More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either alone or in combinations (3). In this regard we have evaluated and compared the antidiabetic potential of Momordica charantia, Picrorrhiza kurroa, Azadirachta indica, Ocimum sanctum and their combination in alloxan induced diabetic rats. All these herbs are reported to possess potent hypoglycaemic activity.

MATERIALS AND METHODS

Drugs, Chemicals and Reagents

Alloxan monohydrate (Spectrochem Pvt. Ltd. Bombay), glibenclamide (Ozone International, Mumbai), accu chek active glucostrips (Roche Diagnostic India Pvt. Ltd., Mumbai) were provided by the central store house, B. R. Nahata College of Pharmacy, Mandsaur.

Extraction

Karela fruit, kutki rhizome, neem leaf and tulsi leaf were collected and extracted using water as the solvent. After extraction the contents were filtered and filtrate was evaporated to dryness.

Procurement and Selection of Animals

Wistar albino rats of either sex weighing between 100 – 150 gm of either sex were obtained from B.R.N.C.P. Mandsaur animal house. These animals were used for the acute toxicity and antidiabetic activity studies. The animals were stabilized for 1 week; maintained in standard condition at room temp; 60 ± 5% relative humidity and 12 h
light dark cycle. They had been given standard pellet diet and water ad-libitum throughout the course of the study. The study was approved by Institutional Animal Ethics Committee (Reg No. 981/ac/05/CPCSEA).

Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No. 420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/kg body weight was adopted. The animals were fasted overnight and next day the extracts (suspended in 5% tween 80 solution) were administered orally at a dose level 2000 mg/kg. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days (4).

Selection of Doses

For the assessment of antidiabetic activity, two dose levels were chosen in such a way that, one dose was approximately one tenth of the maximum dose during acute toxicity studies, and a high dose, which was twice that of one tenth dose (200mg/kg, 400mg/kg).

Antidiabetic Activity

Induction of diabetes:

Overnight fasted albino rats were made diabetic by injecting alloxan monohydrate (in the ice cold normal saline) intraperitoneally at a dose of 120 mg/kg body weight. After that the animals were left aside for 4 hrs and then 10% glucose solution was placed in the cages at a dose of 120 mg/kg body weight. After that the animals were left allloxan monohydrate (in the ice cold normal saline) intraperitoneally. Rats with blood glucose level above 250 mg/dl were aside for 4 hrs and then 10% glucose solution was placed in the cages.

Antidiabetic activity screening in experimentally induced diabetic rats:

The diabetic rats were divided into 13 groups of 6 rats in each. Group I and II served as normal and diabetic control respectively and received vehicle (1 ml/kg, po). Group III received glibenclamide 4 mg/kg. Group IV-XI were treated orally with chosen plant extracts at two dose levels. Group XII and XIII were treated orally with their combination Kr: Kt: N: T (2:1:1:1) at 200 mg/kg and 400 mg/kg respectively. The extracts and glibenclamide were given from 3rd day after alloxanization.

Determination of antidiabetic activity:

The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3rd, 7th and 11th day of the experiment (5, 6, 7). The BGL was determined by the Accu check active glucometer.

Biochemical determinations:

After 11th day of treatment, blood was collected from the retro orbital sinus of overnight fasted rats. The serum was separated and urea, creatinine, triglycerides and cholesterol levels were determined using urea berthelot test kit, creatinine mono reagent test kit, triglycerides test kit and cholesterol test kit, (Span diagnostic Ltd., Surat) respectively.

Statistical Analysis

The data were expressed as mean ± SEM. The data of antidiabetic activity were analyzed by one way analysis of variance (ANOVA) followed by “Dunnett’s test.” P value less than 0.05 was considered as statistically significant.

RESULTS

Acute Toxicity Studies

Acute toxicity studies on female rats showed no mortality at

Table 1: Antidiabetic activity of extracts in alloxan induced diabetic rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Blood glucose level (mg/dl)</th>
<th>0th day</th>
<th>3rd day</th>
<th>7th day</th>
<th>11th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5 % tween 80(1 ml/kg)</td>
<td>83.33 ± 4.12</td>
<td>81.33 ± 2.77</td>
<td>81.00 ± 2.22</td>
<td>79.17 ± 1.80</td>
<td></td>
</tr>
<tr>
<td>Neem extract</td>
<td>200 mg/kg</td>
<td>81.50 ± 4.68</td>
<td>301.17 ± 11.18</td>
<td>180.33 ± 8.26**</td>
<td>144.33 ± 4.51**</td>
<td></td>
</tr>
<tr>
<td>Nimes extract</td>
<td>400 mg/kg</td>
<td>83.33 ± 4.81</td>
<td>307.17 ± 11.18</td>
<td>180.33 ± 8.26**</td>
<td>144.33 ± 4.51**</td>
<td></td>
</tr>
<tr>
<td>Neem extract</td>
<td>200 mg/kg</td>
<td>82.67 ± 2.93</td>
<td>303.33 ± 9.46</td>
<td>207.17 ± 8.00**</td>
<td>161.50 ± 6.96**</td>
<td></td>
</tr>
<tr>
<td>Karel extract</td>
<td>400 mg/kg</td>
<td>83.00 ± 3.42</td>
<td>300.00 ± 13.78</td>
<td>174.17 ± 7.00**</td>
<td>138.17 ± 6.53**</td>
<td></td>
</tr>
<tr>
<td>Neem extract</td>
<td>200 mg/kg</td>
<td>83.17 ± 3.23</td>
<td>301.17 ± 8.80</td>
<td>194.50 ± 9.40**</td>
<td>157.50 ± 6.23**</td>
<td></td>
</tr>
<tr>
<td>Neem extract</td>
<td>200 mg/kg</td>
<td>82.83 ± 4.02</td>
<td>309.00 ± 12.72</td>
<td>234.17 ± 10.04**</td>
<td>181.33 ± 3.94**</td>
<td></td>
</tr>
<tr>
<td>Neem extract</td>
<td>400 mg/kg</td>
<td>82.00 ± 3.65</td>
<td>325.67 ± 27.42</td>
<td>207.00 ± 8.58**</td>
<td>162.67 ± 6.29**</td>
<td></td>
</tr>
<tr>
<td>Kr Kt N T (2:1:1:1)</td>
<td>200 mg/kg</td>
<td>81.83 ± 2.80</td>
<td>298.83 ± 5.22</td>
<td>174.33 ± 2.96**</td>
<td>132.67 ± 5.93**</td>
<td></td>
</tr>
<tr>
<td>Kr Kt N T (2:1:1:1)</td>
<td>400 mg/kg</td>
<td>82.33 ± 2.55</td>
<td>305.83 ± 5.88</td>
<td>157.17 ± 3.26**</td>
<td>118.67 ± 2.75**</td>
<td></td>
</tr>
</tbody>
</table>

Value expressed in mean ± SEM (n=6), *p<0.05, **p < 0.01 v/s Negative control (ANOVA followed by Dunnett’s Test) Kr-Karel, Kt-Kutki, N-Neem, T-Tulsi
Table 2: Effect of Extracts on 11th day on Biochemical Parameters in Alloxan Induced Diabetic Rats (mg/dl)

<table>
<thead>
<tr>
<th>Group</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>41.41+</td>
<td>0.85±0.03</td>
<td>81.80±1.82</td>
<td>99.20±1.71</td>
</tr>
<tr>
<td>Negative control</td>
<td>73.88+</td>
<td>1.65±0.02</td>
<td>173.77±2.55</td>
<td>165.33±2.81</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>28.68±</td>
<td>1.11±0.02</td>
<td>87.25±1.42</td>
<td>67.33±0.70**</td>
</tr>
<tr>
<td>Karela extract</td>
<td>50.77±</td>
<td>1.18±0.03</td>
<td>89.52±1.25**</td>
<td>100.43±1.72**</td>
</tr>
<tr>
<td>Karela extract</td>
<td>39.20±</td>
<td>1.14±0.03</td>
<td>111.85±1.72</td>
<td>98.45±0.50**</td>
</tr>
<tr>
<td>Kutki extract</td>
<td>52.67±</td>
<td>1.23±0.02</td>
<td>95.52±1.76**</td>
<td>107.20±1.42**</td>
</tr>
<tr>
<td>Kutki extract</td>
<td>50.77±</td>
<td>1.18±0.03</td>
<td>89.52±1.25**</td>
<td>100.43±1.71**</td>
</tr>
<tr>
<td>Neem extract</td>
<td>51.45±</td>
<td>1.27±0.02</td>
<td>114.03±3.43</td>
<td>94.07±1.94**</td>
</tr>
<tr>
<td>Neem extract</td>
<td>48.97±</td>
<td>1.22±0.01</td>
<td>102.95±1.58</td>
<td>85.32±1.76**</td>
</tr>
<tr>
<td>Tulsi extract</td>
<td>66.18±</td>
<td>1.21±0.03</td>
<td>98.30±1.16**</td>
<td>84.00±1.47**</td>
</tr>
<tr>
<td>Tulsi extract</td>
<td>61.30±</td>
<td>1.15±0.02</td>
<td>91.35±1.02**</td>
<td>78.27±1.44**</td>
</tr>
<tr>
<td>Kr Kt N T (2:1:1:1) 40.82±0.99**</td>
<td>1.10±0.03</td>
<td>94.33±1.33**</td>
<td>77.82±1.13**</td>
<td></td>
</tr>
<tr>
<td>Kr Kt N T (2:1:1:1) 39.10±0.82**</td>
<td>1.07±0.02</td>
<td>88.93±1.37**</td>
<td>71.27±1.30**</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SEM (n=6), **p < 0.01 vs Negative control (ANOVA followed by Dunnett's Test) Kr-Karela, Kt-Kutki, N-Neem, T-Tulsi

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**Antidiabetic Activity Screening in Experimentally Induced Diabetic Rats**

All the extracts karela, kutki, neem and tulsi showed significant antidiabetic activity at both the dose levels, 200 mg/kg and 400 mg/kg. The combination of Kr Kt N T (2:1:1:1) extracts at a dose of 400 mg/kg showed most significant decrease in blood glucose level, i.e., brought the BGL at near similar to the standard drug glibenclamide (4 mg/kg) on 11th day of the studies. The most effective extracts was found in the order of Kr Kt N T > Kutki > Karela > Neem > Tulsi on the 11th day of study. The results are shown in Table 1.

**Effect on Biochemical Parameters in Alloxan Induced Diabetic Rats**

On estimation of serum biochemical parameters a varying effect was observed. In our study serum urea level was greatly reduced by the karela fruit extract. The most effective extract to reduce urea was found in the order of Karela > Kr Kt N T > Neem > Kutki > Tulsi. Serum creatinine level was most significantly reduced by the Kr Kt N T extract. The most effective extracts to reduce serum creatinine was found in the order of Kr Kt N T > Karela > Tulsi > Kutki > Neem. Kr Kt N T extract most significantly reduce the serum cholesterol level and the most effective extract was found in the order of Kr Kt N T > Kutki > Tulsi > Neem > Karela. Effective extracts in reducing triglycerides level was found in the order of Kr Kt N T > Kutki > Tulsi > Neem > Karela > Kutki. In our study Kr Kt N T maintained the serum urea and cholesterol and karela fruit extract maintained the creatinine level near similar to standard drug glibenclamide. The results are shown in Table 2.

**DISCUSSION**

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The present study was undertaken to develop a new formula that could be used in the treatment of diabetes. A variety of orally active hypoglycaemic agents are frequently used to help manage the glucose intolerance of NIDDM patients. But the effectiveness of these drugs is limited and suffers from a variety of side effects including hypoglycaemia. Many patients develop failure to oral anti-hyperglycaemic agents and consequently need insulin therapy, which has disadvantages of its own (accurate dosing, danger of hypoglycaemia, parenteral therapy and short shelf life). All these factors together reduce compliance. On the other hand plant extracts evaluated in this study are commonly used vegetable and fruits in India and juice of these plants are commonly employed as a household remedy for diabetes (8, 9).

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. The plant extracts and the combination tested for antidiabetic activity exhibited significant activity and effectively reduced the blood sugar level compared to control group. The activities of all the plants tested were well established and they act through different mechanisms (6, 7, 8). The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat deposits mainly due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglycerideremia and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities (10, 11, 12). In our study also the diabetic rats showed hypercholesterolemia and hypertriglycerideremia and the treatment with plant extracts and combination significantly (p<0.05) (p<0.01) decreased both cholesterol and triglyceride levels. This implies that the plant extracts and combination can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycaemia and hypercholesterolemia coexist quite often.
creatinine in the diabetic groups compared to control level. While, after treatment of alloxan-diabetic rats with extracts, the level of urea and creatinine were significantly (p<0.05) (p<0.01) decreased compared to the mean value of diabetic group. This further confirms the utility of these plants and combination in diabetes associated complications (14). Comparatively the combination has produced better results and it may be due to the synergistic effects of extracts when combined together.

CONCLUSION

Our study provides a way to study the activity of individual extracts Vs combination of extracts for the development of polyherbal antidiabetic formulation. This developed formula could do better than using any of the individual drugs mentioned above.

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