HERBAL FORMULATION AND ITS EVALUATION FOR ANTIDIABETIC ACTIVITY

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Summary

The objective of the study is to prepare and investigate the herbal formulation of \textit{Tinospora cordifolia}, \textit{Trigonella foenum} and \textit{Emblica officinalis} for antidiabetic effects.

Herbal formulations PD1, PD2 and PD3 were prepared using \textit{Tinospora cordifolia}, \textit{Trigonella foenum} and \textit{Emblica officinalis} extracts.

Herbal formulations were evaluated for hypoglycemic effects and Oral Glucose Tolerance Test (OGTT) in normal and Alloxan induced diabetic rats.

In hypoglycemic study and OGTT, there was a significant decrease in Blood Glucose Level (BGL) in normal rats with formulation PD3, marginal decrease in formulation PD2 and very less decrease in formulation PD1. In diabetic rats PD3 shown significant decrease in Fasting Blood Glucose Level (FBGL) which was comparable to Glibenclamide while the effects of formulation PD2 and PD1 was not significant after treatment with prepared herbal formulations.

These results were also supported by serum lipid profile and histological studies of liver and kidney.

\textbf{Keywords:} \textit{Tinospora cordifolia}, \textit{Trigonella foenum}, \textit{Emblica officinalis} Hypoglycemic activity, Anti-diabetic activity, Alloxan.

Introduction

It is a general belief that a synergism between two or more plant extracts enhances the physiological potential of the bio-organic substances. Therefore, a combination of different plant extracts is very often preferred over single extracts. Although several reports are available on the effects of different formulations on the regulation of various disorders, only few investigations have been done to study the combined effects of three plant extracts and practically no literature is available with respect to the regulation of hyperglycemia. (Tahiliani and Kar, 2003)

Considering the information gap in this area of research and based on the fact that \textit{Trigonella foenum graecum}, \textit{Emblica officinalis}, \textit{Tinospora cordifolia} extracts were able to ameliorate hyperglycemia in rats individually, a study was made to evaluate possible synergistic effects of these three extracts in single formulation. (Tahiliani and Kar, 2003)
Material and Methods

1 Collection of standardized extracts
Standardized extracts of Tinospora cordifolia was obtained from Himalaya Drug Company and Trigonella foenum and Emblica officinalis were obtained from SAMI Labs, Bangalore (India)

2 Drugs/ chemicals
Tween-80 (Rankem Ranbaxy Fine Chemicals Ltd, New Delhi, India), Glibenclamide Tab. (Aventis Pharma Ltd., Mumbai, India), Alloxan (Spectrochem Pvt. Ltd., Mumbai, India), Glucon D (Heinz India Pvt. Ltd., Mumbai, India), Glucose estimation kit. (Span Diagnostic Ltd., Surat, India)
All the other solvents and chemicals used, were of analytical grade and were purchased from S.D. Fine Chemicals Pvt. Ltd. Mumbai, India

3 Preparation of herbal formulation
All the standardized extracts were properly dried, reduced to fine powder and the powders were sieved through 80 mesh sieve separately. Powder of standardized extracts was weighed accurately for different formulation according to description given below and mixed well together. Thus the Herbal formulation Churna was prepared. Formulations were kept in air tight containers. (Agrawal and Paridhavi, 2007)

PD1 : Tinospora cordifolia : Trigonella foenum : Emblica officinalis (1:1:2)
PD2 : Tinospora cordifolia : Trigonella foenum : Emblica officinalis (1:2:1)
PD3 : Tinospora cordifolia : Trigonella foenum : Emblica officinalis (2:1:1)

4 Dose fixation 100, 200, 300 and 400 mg/kg doses were tried at first, among of these 100 and 200 mg/kg doses did not show significant reduction in blood glucose level in alloxan induced diabetic rats, 300 mg/kg showed a moderate antidiabetic effect while 400 mg/kg showed significant reduction in blood glucose level. So all our study was carried out with 400 mg/kg dose level.

5 Animals
Albino Wistar rats (150-200 g) were used for the study. The animals had free access to standard rat pellet (Pranav Agro Industries Ltd, Bangalore, India), with water supplied ad libitum and kept under strict hygienic standard conditions.
All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

6 Preparation of drugs:
Standardized extracts of Trigonella foenum, Tinospora cordifolia and Emblica officinalis suspended in water in presence of 3% v/v Tween-80 solution.
All the drugs were administered orally for experimental purpose. Each time fresh preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10 ml/kg for each animal.

Glibenclamide Solution: Glibenclamide tablet was dissolved in 1ml of distilled water to give 500 µg/ml solution. This solution was administered at a dose of 10mg/ kg body weight using clean and dry oral feeding needle for 21days.
7 Experimental antidiabetic models followed in the present work
Hypoglycemic studies in normal rats
Oral glucose tolerance test in normal rats
Anti diabetic studies in Alloxan induced diabetic rats
Oral glucose tolerance test in Alloxan induced diabetic rats
Study of lipid profile
Study of histological changes in liver and kidney

8 Statistical Analysis
The values were expressed as mean ± SEM. Statistical comparisons between all groups were performed by using ANOVA-1.

Results

1 Hypoglycemic activity in normal rats
Among herbal formulations PD1, PD2 and PD3 only PD3 significantly decreased the blood glucose level on 7th day after treatment. Glibenclamide (10 mg/kg) significantly reduced blood glucose level (BGL) on 7th day as compare to vehicle and normal groups. (Table No: 1) (Kesari et al., 2006 and Gupta et al., 2005)

2 Oral glucose tolerance test (OGTT) on 8th day:
Administration of glucose (2gm/kg) to 7 days pretreated rats significantly suppress the rise in BGL with PD3 at 1 hour and 2 hour with 400 mg/kg as compare with vehicle control. While in PD1 (400 mg/kg) did not produce significant reduction in BGL. Glibenclamide (10 mg/kg) showed significant suppress in BGL rise at 1 & 2 hour. (Table No: 3)

Table No: 1 Hypoglycemic activity in normal rats after 7 days treatment with herbal formulations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose level(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>---</td>
<td>98.43 ± 2.16</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>10</td>
<td>93.79 ± 2.58</td>
</tr>
<tr>
<td>STD</td>
<td>10</td>
<td>97.43 ± 3.66</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>98.74 ± 2.09</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>98.07 ± 5.29</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>94.38 ± 3.82</td>
</tr>
</tbody>
</table>

Table No: 2 Antidiabetic activity in alloxan induced diabetic rats after 21 days treatment with herbal formulations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood Glucose Level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 Day</td>
</tr>
<tr>
<td>Normal Control</td>
<td>---</td>
<td>83.16 ± 1.95</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>10</td>
<td>322.33 ± 11.16</td>
</tr>
<tr>
<td>STD</td>
<td>10</td>
<td>260.33 ± 3.88</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>270.33 ± 6.80</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>266.33 ± 8.67</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>269.5 ± 8.80</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with
+ P <0.05 and ++ P <0.01 Normal Control Vs all groups.
* P <0.05 and ** P <0.01 Diabetic Control Vs all group

Treatment with alloxan (120 mg/kg, i.p.) increased the BGL to a range of 260-325 mg/dl after 7 days. (Hayashi et al., 2002)
Treatment with herbal formulation PD3 (400 mg/kg) had significantly reduced the BGL on 12th and 21st day in alloxan induced diabetic rats. PD2 (400 mg/kg) significantly reduced BGL on 21st day in alloxan induced diabetic rats, indicating the formulation PD2 possess anti-diabetic activity after 3 weeks. Whereas, anti-diabetic activity is significant on 12th and 21st day for glibenclamide treated groups as compare with diabetic control groups. (Table No: 2) (Ghosh, 2001 and Sharma, 2006)

4 Oral Glucose Tolerance Test (OGTT) in alloxan induced diabetic rats on 22nd day:
Repeated administration of herbal formulation PD3 and PD2 (400 mg/kg), and Glibenclamide (10 mg/kg) significantly inhibited the increase in BGL at 1st, 2nd and 3rd hour after glucose loading (2 g/kg) in alloxan induced diabetic rats. (Table No: 4)

5 Serum Lipid Profile
Treatment of PD3 significantly lowered serum total cholesterol, triglycerides and significantly elevated HDL level compared to the positive control group. (Table No: 5) (Naveen et al., 2007; Kannur et al 2006)

6 Histopathology of Liver after 21 Days of Treatment (Fig No:1)
Normal- Histology of the liver sections of normal control animals showed normal liver architecture with well brought out central vein, well-preserved cytoplasm and prominent nucleus and nucleolus.
Diabetic Control- The alloxan-induced diabetic rat displayed feathery degeneration, micro and macro cellular fatty changes and inflammatory cells around portal tract.
STD (Glibenclamide)- Glibenclamide treated animals showed an mild protection from alloxan-induced changes in the liver.
PD1- This group showed mild inflammatory infiltration filling over the sinusoidal vacuolation of hepatocytic nuclei.
PD2- Less micro and macro cellular fatty changes in compare to diabetic control group.
PD3- No fatty degeneration and showed good protection against alloxan induced toxicity.

7 Histopathology of Kidney after 21 Days of Treatment (Fig No:2)
Normal- Histology of the kidney sections of normal control animals showed normal kidney architecture with well brought glomeruli and tubules. Well-preserved cytoplasm, prominent nucleus and nucleolus.
Diabetic Control- The alloxan-induced diabetic rat, displayed feathery degeneration, thickening of glomeruli, inflammatory cells & severe congestion.
STD (Glibenclamide)- Glibenclamide treated animals showed protection from alloxan-induced changes in the kidney. Mild inflammatory changes were noticed here.

PD1- It showed mild protection from alloxan treated groups. Mild congestion was noticed here.

PD2- Mild inflammation, atropic changes of glomeruli cells were observed. It showed mild protection.

PD3- Mild congestion and hypertrophy of glomeruli were observed but compare to diabetic control it was found less.

Discussion

In light of the above reports, an attempt was made to study the synergistic effect in the different combinations of extracts of *Tinospora cordifolia*, *Trigonella foenum* and *Emblica officinalis* in herbal formulation.

The standard drug glibenclamide (10 mg/kg) treated group has shown significant decrease in fasting glucose level and serum lipid profile in comparison to the diabetic control group. (Yesilada et al., 1999)

Herbal formulation PD3 produced a statistically significant decrease in blood glucose levels for both normoglycaemic and alloxan induced hyperglycaemic rats.

Formulation PD2 showed significant reduction in blood glucose level in alloxan induced hyperglycaemic rats, only on 21st day while herbal formulation PD1 did not show significant reduction in blood glucose level. Alloxan selectively destroys insulin secreting β-cells in the islets of Langerhans and the effect is irreversible. Alloxan treated animals receiving the herbal formulation PD3 showed rapid normalization of blood glucose level in comparison to the control and this could be due to the possibility that many β-cells are still surviving and cell regeneration can not be ignored and the reductions in the serum glucose levels may be due to the increase in action of GLUT4 receptors or insulinomimetic action as per previous reports. (Liu et al., 2005; Judy et al., 2003 and Custer, 2005)

Lipid profiles of animals treated with herbal formulations were studied. Treatment with herbal formulation PD2 and PD3 significantly lowered serum total cholesterol. The same effect was noticed with triglycerides LDL and VLDL; while HDL levels were increased due to the treatment with herbal formulation. (Sinha, 1972)

Histopathological studies revealed that glibenclamide and herbal formulations treated groups shown hepatoprotective and nephroprotective effect against oxidative stress compared to diabetic control group. Diabetic control group shown the symbol of nephrotoxicity and hepatic-injury due to oxidative stress.

The study of blood glucose levels, lipid profile and histological changes in liver and kidney, in herbal formulations treated rats, support the Herbal formulation PD3 is a potent antidiabetic.
Fig. No: 1 Histopathology of Liver after 21 Days of Treatment

Fig. No: 2 Histopathology of Kidney after 21 Days of Treatment
Table No: 3 Oral Glucose Tolerance Test in normal rats on 8th day, after treatment with herbal formulations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 hour</th>
<th>1st hour</th>
<th>2nd hour</th>
<th>3rd hour</th>
<th>6th hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BGL</td>
<td>% change</td>
<td>BGL</td>
<td>% change</td>
<td>BGL</td>
</tr>
<tr>
<td>Normal Control</td>
<td>-</td>
<td>97.56</td>
<td>±</td>
<td>149.08</td>
<td>±</td>
<td>117.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.65</td>
<td>2.53</td>
<td>±</td>
<td>3.60</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97.70</td>
<td>±0.94</td>
<td>2.63</td>
<td>±</td>
<td>3.66</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>10</td>
<td>97.70</td>
<td>±0.94</td>
<td>2.63</td>
<td>±</td>
<td>3.66</td>
</tr>
<tr>
<td>STD Glibenclamide</td>
<td>10</td>
<td>50.98</td>
<td>±2.23</td>
<td>3.47</td>
<td>±</td>
<td>3.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.98</td>
<td>±2.23</td>
<td>3.47</td>
<td>±</td>
<td>3.66</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>94.85</td>
<td>±2.09</td>
<td>3.12</td>
<td>±</td>
<td>3.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.09</td>
<td>3.12</td>
<td>±</td>
<td>3.23</td>
<td>±</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>89.00</td>
<td>±2.37</td>
<td>3.64</td>
<td>±</td>
<td>2.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.37</td>
<td>3.64</td>
<td>±</td>
<td>2.42</td>
<td>±</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>73.16</td>
<td>±2.33</td>
<td>2.42</td>
<td>±</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±2.33</td>
<td>2.42</td>
<td>±</td>
<td>2.64</td>
<td>±</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with 
+ P <0.05, ++ P <0.01 Normal Control Vs all groups. * P <0.05, ** P <0.01 Vehicle Control Vs all groups.

% change means, percentage increase in BGL after glucose (2 g/kg, p.o.) administration.
Table No: 4 Oral Glucose Tolerance Test in alloxan induced diabetic rats on 22\textsuperscript{nd} day, after treatment with herbal formulations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 hour</th>
<th>1\textsuperscript{st} hour</th>
<th>2\textsuperscript{nd} hour</th>
<th>3\textsuperscript{rd} hour</th>
<th>6\textsuperscript{th} hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BGL</td>
<td>BGL % change</td>
<td>BGL % change</td>
<td>BGL % change</td>
<td>BGL % change</td>
</tr>
<tr>
<td>Normal Control</td>
<td>---</td>
<td>95.87 ± 3.02</td>
<td>134.43 ± 0.99</td>
<td>112.20 ± 2.70</td>
<td>98.07 ± 0.51</td>
<td>96.73 ± 1.22</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>10</td>
<td>283.42 ± 3.20</td>
<td>418.68 ± 5.52</td>
<td>353.01 ± 4.47</td>
<td>297.33 ± 6.08</td>
<td>286.44 ± 4.09</td>
</tr>
<tr>
<td>STD Glibenclamide</td>
<td>10</td>
<td>102.48 ± 0.18</td>
<td>132.16 ± 2.04</td>
<td>114.67 ± 3.48</td>
<td>104.86 ± 2.62</td>
<td>103.60 ± 2.43</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>187.50 ± 1.94</td>
<td>262.93 ± 3.07</td>
<td>225.63 ± 3.83</td>
<td>194.64 ± 1.22</td>
<td>190.29 ± 3.08</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>141.18 ± 3.50</td>
<td>193.61 ± 2.86</td>
<td>162.80 ± 0.93</td>
<td>144.86 ± 2.88</td>
<td>142.87 ± 1.95</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>112.19 ± 4.60</td>
<td>148.47 ± 4.18</td>
<td>127.45 ± 5.29</td>
<td>114.99 ± 1.73</td>
<td>113.44 ± 2.77</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with
+ $P < 0.05$, ++ $P < 0.01$, Normal Control Vs all groups. * $P < 0.05$, ** $P < 0.01$, Diabetic Control Vs all groups.

% change means, percentage increase in BGL after glucose (2 g/kg, p.o.) administration.
**Table No: 5 Serum Lipid Profile.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/ kg)</th>
<th>Total cholesterol (Mean ± S.E.M.)</th>
<th>Serum Triglyceride (Mean ± S.E.M.)</th>
<th>HDL (Mean ± S.E.M.)</th>
<th>LDL (Mean ± S.E.M.)</th>
<th>VLDL (Mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>---</td>
<td>87.02 ± 1.69</td>
<td>93.77 ± 3.90</td>
<td>42.1 ± 0.87</td>
<td>36.05 ± 0.37</td>
<td>19.44 ± 1.98</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>10</td>
<td>125.88 ± 7.22</td>
<td>163.32 ± 12.31</td>
<td>35.7 ± 1.38</td>
<td>54.12 ± 2.70</td>
<td>23.62 ± 1.30</td>
</tr>
<tr>
<td>STD Glibenclamide</td>
<td>10</td>
<td>73.99 ± 10.17</td>
<td>61.33 ± 9.75</td>
<td>45.13 ± 0.87</td>
<td>38.65 ± 2.78</td>
<td>20.88 ± 2.18</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>99.56 ± 6.57</td>
<td>133.70 ± 3.66</td>
<td>39.17 ± 0.27</td>
<td>48.44 ± 3.20</td>
<td>23.09 ± 2.25</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>87.12 ± 3.43</td>
<td>119.60 ± 4.96</td>
<td>42.11 ± 0.86</td>
<td>42.44 ± 7.45</td>
<td>22.45 ± 1.11</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>79.71 ± 4.12</td>
<td>97.42 ± 7.54</td>
<td>44.12 ± 0.37</td>
<td>40.05 ± 5.29</td>
<td>21.03 ± 1.65</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with
+ P <0.05, ++ P <0.01, Normal Control Vs all groups. * P <0.05, ** P <0.01, Diabetic control Vs all group
References


**Acknowledgement**

This dissertation is built on Herbal formulation and its evaluation for anti-diabetic activity in alloxan induced diabetic rats. The work with this dissertation has been extensive and trying, but in the first place exciting, instructive and fun. without help, support and encouragement from several persons, I would never have been able to finish this work.

I would like to express my gratitude to Professor Dr. S. Mohan (Principal and Director), PES College of Pharmacy for his generous consideration and facilities provided.

It gives a great pleasure to acknowledge my immense respect and depth of gratitude to my esteemed guide Mr. G. Murugananthan who has been a constant source of encouragement and treasure of valuable inspiring guidance.

I express my sincere thanks to Himalaya Drug Company and SAMI Labs for providing standardized extracts and Dr. L. Krishna (Medical Superintendent), Dr. A. S. Ramaswamy (M.D. & Asst. Prof.), Dr. Yenni (H.O.D. Dept. of Pathology), Dr. H.K. Manjunath (M.D. & Asst. Prof.); PES Institute of Medical Sciences & Research, Kuppam; for helping me to successfully complete my research work.

It is indeed a difficult task to acknowledge the services of all those who have extended their valuable assistance directly or indirectly. I sincerely thanks to them all.