Herbal Formulation and Its Evaluation for Antidiabetic Activity

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ABSTRACT

The objective of the study is to prepare and investigate the herbal formulation of *Tinospora cordifolia, Trigonella foenum* and *Emblica officinalis* for antidiabetic effects. All standardized extracts of *Tinospora cordifolia, Trigonella foenum* and *Emblica officinalis* were subjected to preliminary phytochemical evaluation. In chemical evaluation of extracts, it showed that *Tinospora cordifolia* contains alkaloid, glycoside, phytosterols, phenolics and tannins, proteins and amino acids, fixed oils and fats, carbohydrates; *Trigonella foenum* contains alkaloid, glycoside, saponins, proteins and amino acids, carbohydrates and *Emblica officinalis* contains alkaloid, glycoside, phytosterols, phenolics and tannins, proteins and amino acids, carbohydrates as a constituents. Herbal formulations PD1, PD2 and PD3 were prepared using *Tinospora cordifolia, Trigonella foenum* and *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.


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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\(^1\). Considering the information gap in this area of research and based on the fact that *Trigonella foenum graecum*, *Embelica officinalis*, *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\(^1\).

MATERIAL AND METHODS

COLLECTION OF STANDARDIZED EXTRACTS

Standardized extracts of *Tinospora cordifolia* was obtained from Himalaya Drug Company and *Trigonella foenum* and *Embelica officinalis* were obtained from SAMI Labs, Bangalore (India).

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

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\(^2-5\).

PD1 : *Tinospora cordifolia* : *Trigonella foenum* : *Embelica officinalis* (1:1:2)

PD2 : *Tinospora cordifolia* : *Trigonella foenum* : *Embelica officinalis* (1:2:1)


Four 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.

**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.

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of PES College of Pharmacy, Bangalore 560050. (Letter No- *PESCP/IAEC/07/15*), CPCSEA Registration No. 836/AC/04/CPCSEA

**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.

**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

**ASSESSMENT OF HYPOGLYCEMIC ACTIVITY IN NORMAL RATS:**

Normal rats were tested for the hypoglycemic effects of the various formulations of standardized extracts.

**Animals:**

Albino Wistar rats of either sex weighing 150-200 gm were divided into 6 groups consisting of 6 rats in each group.
Groups:

Group 1: Normal control received distilled water (10 ml/kg, p.o.)

Group 2: Vehicle control received 3% v/v Tween 80 in water (10 ml/kg, p.o.)

Group 3: Standard group received Glibenclamide (10 mg/kg, p.o.)

Group 4: PD1 group received formulation 1 (400 mg/kg, p.o.)

Group 5: PD2 group received formulation 2 (400 mg/kg, p.o.)

Group 6: PD3 group received formulation 3 (400 mg/kg, p.o.)

Procedure:

All the animals were grouped randomly into six groups of six in each and were fasted over night. The blood samples were withdrawn from retro orbital plexus at 0 hour i.e. just prior to oral administration of herbal formulation and collected blood samples were subjected to cool centrifugation for serum separation. Serums were separated and treated with GOD/POD kit and according to procedure blood glucose levels were determined under U.V. spectrophotometry. The administration of drug was continued for next 21 days. BGL was estimated on 7th day after 1 hour of drug administration.

ORAL GLUCOSE TOLERANCE TEST (OGTT) IN NORMAL RATS:

On the next day (8th day) after the assessment of hypoglycemic activity OGTT was carried out for normal animals.

Procedure:

After 60 min of drug administration, the rats of all the groups were orally treated with 2 g/kg of glucose. The blood samples were collected from retro orbital plexus at 0 hour i.e. just prior to the administration of glucose load and at 1st hour, 2nd hour, 3rd hour and 6th hour after the administration of the glucose load. Blood glucose level was estimated at various time intervals.

ASSESSMENT OF ANTI-DIABETIC ACTIVITY IN ALLOXAN INDUCED DIABETIC RATS:

Induction of Diabetes:

Albino Wistar rats of either sex weighing 170-220 g were selected for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages. Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan. After alloxan injection rats were given 10% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than...
200 mg/dl were selected and used for further observation. All the animals were allowed free access to water and standard pellet diet and maintained at room temperature.

All the animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser then 400 mg/dl) and such animals were selected and divided into groups of six each and used for the study of the following experimental models:

**Groups:**

**Group 1:** Normal control received distilled water (10 ml/kg, p.o.)

**Group 2:** Diabetic control received 3% v/v Tween 80 in water (10 ml/kg, p.o.)

**Group 3:** Standard group received Glibenclamide (10 mg/kg, p.o.)

**Group 4:** PD1 group received formulation 1 (400 mg/kg, p.o.)

**Group 5:** PD2 group received formulation 2 (400 mg/kg, p.o.)

**Group 6:** PD3 group received formulation 3 (400 mg/kg, p.o.)

**Procedure:**

Animals were grouped randomly into six groups of six each and were fasted over night. Drug treatment was made as mentioned above. The blood samples were withdrawn from retro orbital plexus at 0 hour i.e. just prior to oral administration of all the drugs. The treatment was continued for next 21 days. The blood samples were also withdrawn on 12th and 21st day after 1 hour administration of drug. Blood glucose level was estimated on various day intervals.

**ORAL GLUCOSE TOLERANCE TEST (OGTT) IN ALLOXAN INDUCED DIABETIC RATS:**

On 22nd day OGTT is carried out in the same alloxan induced rats using for assessment anti-diabetic activity studies.

**Procedure:**

After 60 min of drug administration, the rats of all the groups, were orally treated with 2 g/kg of glucose. The blood samples were collected from retro orbital plexus at 0 hour i.e. just prior to the administration of glucose load and at 1st hour, 2nd hour, 3rd hour and 6th hour after the administration of the glucose load. Blood glucose level was estimated at various time intervals.

**ESTIMATION OF SERUM GLUCOSE:**

Span diagnostics kit was used for the estimation of serum glucose. Estimation was carried out by GOD/POD method.
The enzyme glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide as follows:

\[
\text{Glucose + O}_2 + \text{H}_2\text{O} \rightarrow \text{Gluconic acid + H}_2\text{O}_2
\]

Addition of the enzyme peroxidase and a chromogen such as 4 Aminoantipyrine results in the formation of a colored compound quinoneimine that can be measured.

\[
\text{2H}_2\text{O}_2 + 4\text{-Aminoantipyrine phenol} \rightarrow \text{Quinonimine + 4H}_2\text{O}
\]

The red color of Quinoneimine is measured at 505 nm and is directly proportional to glucose concentration. Glucose oxidase is highly specific for β-D glucose because 36% and 64% of glucose in solution are in alpha and beta forms respectively, complete reaction requires mutarotation of the α to β form. The second step, involving peroxidase, is much specific than the glucose oxidase reaction. Various substances, such as uric acid, ascorbic acid, bilirubin, haemoglobin, tetracycline and glutathione, inhibit the reaction (presumably by competing with chromogen for H\textsubscript{2}O\textsubscript{2}) producing lower values. Some glucose oxidase preparations contain catalase as a contaminant; catalase activity decomposes peroxide and decreases the intensity of the final color obtained.

**HISTOPATHOLOGY:**

Histopathological examination was done for right kidney and one lobe of the liver. Depending on the treatment protocol the extent of hemorrhage was examined using electron micro-scope.

**Serum lipid profile:**

Total Cholesterol, Triglycerides, HDL, LDL, VLDL was measured from the collected serum samples on 21\textsuperscript{st} day after treatment.

**Statistical Analyses:**

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

**RESULTS**

**Hypoglycemic activity in normal rats**

Among herbal formulations PD1, PD2 and PD3 only PD3 significantly decreased the blood glucose level on 7\textsuperscript{th} day after treatment. Glibenclamide (10 mg/kg) significantly reduced blood glucose level (BGL) on 7\textsuperscript{th} day as compare to vehicle and normal groups (Table No: 1)
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>
<th>0 Day</th>
<th>7th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>---</td>
<td>98.43 ± 2.16</td>
<td>97.30 ± 3.65</td>
<td></td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>10</td>
<td>93.79 ± 2.58</td>
<td>93.82 ± 2.35</td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>10</td>
<td>97.43 ± 3.66</td>
<td>48.28#** ± 4.27</td>
<td></td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>98.74 ± 2.09</td>
<td>90.06 ± 2.32</td>
<td></td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>98.07 ± 5.29</td>
<td>81.85 ± 6.65</td>
<td></td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>94.38 ± 3.82</td>
<td>71.27#** ± 3.18</td>
<td></td>
</tr>
</tbody>
</table>

**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: 2)

**Anti-diabetic activity in alloxan induced diabetic rats.**
Treatment with alloxan (120 mg/kg, i.p.) increased the BGL to a range of 260-325 mg/dl after 7 days. 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: 3)

**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)
Table No: 2 Oral Glucose Tolerance Test in normal rats on 8\textsuperscript{th} 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>Normal Control</td>
<td>-</td>
<td>97.5 ± 0.65</td>
<td>149.0 ± 2.53</td>
<td>52.80 ± 3.73</td>
<td>117.6 ± 3.60</td>
<td>20.55 ± 2.20</td>
</tr>
<tr>
<td>Vehicle Control</td>
<td>10</td>
<td>97.7 ± 0.94</td>
<td>148.8 ± 2.63</td>
<td>52.30 ± 3.76</td>
<td>116.8 ± 3.66</td>
<td>19.54 ± 2.19</td>
</tr>
<tr>
<td>STD Glibenclamide</td>
<td>10</td>
<td>50.9 ± 2.23</td>
<td>63.99 ± 2.52</td>
<td>25.51 ± 3.47</td>
<td>52.71 ± 3.72</td>
<td>3.39 ± 1.14</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td>94.8 ± 2.09</td>
<td>134.5 ± 3.12</td>
<td>41.84 ± 1.12</td>
<td>108.1 ± 3.23</td>
<td>14.03 ± 2.51</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td>89.0 ± 2.37</td>
<td>122.1 ± 1.64</td>
<td>37.22 ± 4.64</td>
<td>99.01 ± 2.42</td>
<td>11.24 ± 2.19</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td>73.1 ± 2.33</td>
<td>100.0 ± 2.42</td>
<td>36.79 ± 1.46</td>
<td>80.09 ± 2.64</td>
<td>9.47 ± 1.87</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: 3 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>
<th>0 Day</th>
<th>12th Day</th>
<th>21st Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>---</td>
<td></td>
<td>83.16 ± 1.95</td>
<td>84.02 ± 1.17</td>
<td>82.33 ± 2.17</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>10</td>
<td></td>
<td>322.33 ± 11.16</td>
<td>268.32 ± 10.02</td>
<td>272.83 ± 14.04</td>
</tr>
<tr>
<td>STD</td>
<td>10</td>
<td></td>
<td>260.33 ± 3.88</td>
<td>112.14 ± 1.48</td>
<td>100.83 ± 4.86</td>
</tr>
<tr>
<td>PD1</td>
<td>400</td>
<td></td>
<td>270.33 ± 6.80</td>
<td>224.01 ± 1.60</td>
<td>190.67 ± 8.21</td>
</tr>
<tr>
<td>PD2</td>
<td>400</td>
<td></td>
<td>266.33 ± 8.67</td>
<td>193.00 ± 1.84</td>
<td>146.00 ± 2.30</td>
</tr>
<tr>
<td>PD3</td>
<td>400</td>
<td></td>
<td>269.5 ± 8.80</td>
<td>168.21 ± 1.23</td>
<td>108.83 ± 3.12</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n = 6.
Significant values were compared with

\[ P < 0.05 \text{ and } ** P < 0.01 \text{ Normal Control Vs all groups.} \]

\[ * P < 0.05 \text{ and } ** P < 0.01 \text{ Diabetic Control Vs all group} \]

Table No: 4 Oral Glucose Tolerance Test in alloxan induced diabetic rats on 22nd day, after treatment with herbal formulations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose level (mg/dl) and Percentage change in Blood glucose level (%)</th>
<th>0 hour BGL</th>
<th>1st hour BGL</th>
<th>% change</th>
<th>2nd hour BGL</th>
<th>% change</th>
<th>3rd hour BGL</th>
<th>% change</th>
<th>6th hour BGL</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>---</td>
<td></td>
<td>95.87 ± 3.02</td>
<td>134.43 ± 0.99</td>
<td>40.22 ± 2.29</td>
<td>112.20 ± 2.70</td>
<td>17.03 ± 1.38</td>
<td>98.07 ± 0.51</td>
<td>2.29 ± 0.51</td>
<td>96.73 ± 1.22</td>
<td>0.89 ± 0.36</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>10</td>
<td></td>
<td>283.4 ± 5.20</td>
<td>418.68 ± 3.06</td>
<td>47.72 ± 3.06</td>
<td>353.01 ± 4.47</td>
<td>24.55 ± 1.28</td>
<td>297.33 ± 6.08</td>
<td>4.90 ± 1.20</td>
<td>286.44 ± 4.09</td>
<td>1.06 ± 0.61</td>
</tr>
<tr>
<td>STD Glibenclamide</td>
<td>10</td>
<td></td>
<td>102.4 ± 2.04</td>
<td>132.16 ± 1.90</td>
<td>28.96 ± 3.48</td>
<td>114.67 ± 1.41</td>
<td>11.89 ± 2.62</td>
<td>104.86 ± 2.32</td>
<td>2.32 ± 0.54</td>
<td>103.60 ± 2.43</td>
<td>1.09 ± 0.46</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
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)

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.

![Fig No: 1 Histopathology of Liver after 21 Days of Treatment](image)

**STD (Glibenclamide)**- Glibenclamide treated animals showed a 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.
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.

Fig. No: 2 Histopathology of Kidney after 21 Days of Treatment

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.9

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 reports10-12.

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 formulation13.

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 has 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.

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REFERENCE