Pre-Clinical Evaluation of Anti-Diabetic Activity of Phyllanthus Reticulatus Fruit Extract

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Abstract

Anti-diabetic activity of Phyllanthus Reticulatus fruit extract was carried out against streptozotocin induced diabetic model in wistar rats. Soxhlet extraction using methanol was carried out on Phyllanthus Reticulatus fruit to obtain the extract. Acute Oral Toxicity- Acute Toxic Class Method 423 was performed to obtain the low and high doses respectively at 300 mg/kg and 600 mg/kg. Experiment using streptozotocin as diabetic model was actioned. The parameters which were evaluated were body weight, blood glucose, SOD (superoxide dismutase), CAT (catalase), GSH (glutathione), MDA (malondialdehyde), Triglyceride (TG), Total cholesterol (TC), HDL (High-density lipoprotein) and LDL (Low-density lipoprotein). Streptozotocin brought derangements in the above estimated parameters which were ameliorated by administration of Phyllanthus Reticulatus fruit extract. The findings of the present study support anti-diabetic activity of Phyllanthus Reticulatus fruit extract in the animal model.

Keywords: Anti-Diabetic, Streptozotocin, Phyllanthus Reticulatus, Rats

Introduction

Diabetes mellitus is derived from the Latin term mellitus, which means sweet, and the Greek word diabetes, which means to syphon or flow through. Diabetes mellitus (DM) is a metabolic condition marked by unnecessarily high blood glucose levels. Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) are the two primary subtypes of DM, and both are often brought on by faulty insulin production (T1DM) and/or action (T2DM).¹ ¹ in 10 individuals (20-79 years old) have diabetes, which affects 537 million people. By 2030, this figure is expected to reach 643 million, and by 2045, it will reach 783 million. More than 80% of diabetic individuals reside in low- and middle-income nations. In 2021, diabetes will be the cause of 6.7 million fatalities, or one every five seconds.² One in 12 persons, or about 74 million adults, in India have diabetes. India has the second highest-rate of diabetes cases in the world. In India, there are more than half (53.1%) of diabetics who are undiagnosed. It is estimated that up to 79.4 million people in India might have diabetes mellitus by 2030.³

The majority of treatment for T1DM is insulin delivery by daily injections or an insulin pump because the condition is predominantly brought on by a lack of insulin. For T2DM, the particular drug subtypes include sulfonylureas, biguanides, thiazolidinediones, meglitinides, alpha-glucosidase inhibitors etc. Hypoglycemia is one of the most frequent side effects of insulin. The most frequent negative effect of several T2DM medicines is digestive problems. Lactic acidosis can be brought on by metformin. Cardiovascular death may be encouraged by sulfonylureas. Due to its negative effects, particularly fluid retention that worsens heart failure, thiazolidinediones have lost popularity in clinical practise.⁴

The Latin word "herba" and the ancient French word "herbe" are the origins of the term "herb." Long before the prehistoric era, people employed plants for medical purposes. There is evidence that around 4000 years ago, Unani Hakims, Indian Vaids, and cultures from the Europe used herbs as medicine.⁵ Traditional medical systems are still used extensively on many fronts. The use of plant materials as a source of medicines for a wide range of human ailments has received more attention as a result of factors including population growth,
insufficient drug supply, prohibitive cost of treatments, side effects of several synthetic drugs, and development of resistance to currently used drugs for infectious diseases.[4] Till now, no scientific work has been done on anti-diabetic potential of Phyllanthus Reticulatus fruit extract. Hence the present study is aimed to investigate the anti-diabetic activity of Phyllanthus Reticulatus fruit extract.

2. Materials and Method

Chemicals
Chemicals and solvents used in the present study were obtained from Hi Media Laboratories- Mumbai and Sigma Aldrich India- Bangalore. Biochemical estimation kits were obtained from Crest- Goa.

Animals
or anti-diabetic study, Wistar rats (200g -250g) were weighed and selected. For dose selection study, according to OECD 423 guidelines, Swiss albino mice was used. They all were kept in polypropylene cages. 12 hours’ dark-light cycle, 25±5°C temperature with humidity 50±5% were maintained. Animals were fed a regular pellet diet and had unrestricted access to consuming water at will. Institutional Animals Ethics Committee (IAEC) approved the study [NCPT/IAEC-009/2022]. CPCSEA guidelines and IAEC recommendations were followed throughout the study.

Plant Material and Extraction
The Phyllanthus reticulatus fruits was collected from Uttar Pradesh. The fruits were suitable identified by botanist [APM/22-02-22/01]. Then they were pulverized to obtain powdered product. Extraction was carried out by Soxhlet extraction. Powdered product was extracted in a Soxhlet apparatus using methanol as solvent system. The resultant extracted product was filtered using Watman Filter paper and then dried to remove the solvent part. The dried extract was stored in desiccator for further use. [5]

Dose Selection
The OECD 423 Guidelines were followed for conducting the dose selection research. Here, female Swiss albino mice were divided into 2 groups: 300 mg/kg and 2000 mg/kg groups each consisted 3 animals. According to the aforementioned dosages, the test medication, Phyllanthus Reticulatus fruit extract, was once given. Physical factors such as, any, alterations in the hair, skin, mucous membranes, respiratory, circulatory, autonomic nervous system, central nervous system, and behaviour as well as observations of tremors, convulsions, salivations, diarrhoea, lethargy, sleep, and coma were noted during this time. The test medication failed to exhibit adverse symptoms during 14 day period. The lower and higher dosages were set at 300 mg/kg and 600 mg/kg body weight, respectively, depending on the research.[6]

Streptozotocin Induced Diabetes Mellitus[7]
Wistar male rats was divided into 5 groups of 6 animals each. Group 1 served as normal control and received regular rat food, drinking water at libitum. Groups 2 to 5 overnight fasted animals were administered single intraperitoneal injection of 60 mg/kg STZ dissolved in 0.1 M cold sodium citrate buffer at PH 4.5. Fasting blood glucose level was estimated after 72 hours. Rats showing blood glucose levels more than 250 mg/dl was included in the study.

Group 1: NORMAL [Normal control]
Group 2: DISEASED [Diseased control (Only steptozotocin)]
Group 3: LOW DOSE [Low dose of test drug Phyllanthus Reticulatus fruit extract (300 mg/kg) + Streptozotocin]
Group 4: HIGH DOSE [High dose of test drug Phyllanthus Reticulatus fruit extract (600 mg/kg) + Streptozotocin]
Group 5: STANDARD [Standard anti diabetic drug, Metformin (100 mg/kg) + Streptozotocin]
Treatment went on for 2 weeks. On 15th day animals were sacrificed.

The parameters which were evaluated were body weight, blood glucose, SOD[8] (superoxide dismutase), CAT[9] (catalase), GSH[10] (glutathione), MDA[11] (malondialdehyde), Triglyceride (TG), Total cholesterol (TC), HDL (high-density lipoprotein) and LDL (low-density lipoprotein). Blood glucose, TG, TC, HDL, LDL were estimated using biochemical estimation kits obtained from Crest- Goa.

Statistical Analysis
Statistical analysis was done using Graph Pad Prism Software version 4 [Graph Pad Inc., USA]. One Way Anova followed by Bonferroni’s Test was applied. Confidence interval was taken at 95%.

3. Results and Discussion
Streptozotocin brought extremely significant decrease in body weight [131 ± 0.62] when compared with normal animals [220 ± 0.37]. Treatment with low dose [202 ± 0.71] and high dose [206 ± 0.92] of extract brought extremely significant increase in body weight when compared with diseased control [131 ± 0.62].
Streptozotocin brought extremely significant increase in blood glucose level \([331 \pm 0.46]\) when compared with normal animals \([110 \pm 0.47]\). Treatment with low dose \([139 \pm 0.61]\) and high dose \([131 \pm 0.51]\) of extract brought extremely significant decrease in blood glucose level when compared with diseased control \([331 \pm 0.46]\). Streptozotocin brought extremely significant decrease in SOD level \([21 \pm 0.79]\) when compared with normal animals \([103 \pm 0.74]\). Treatment with low dose \([90 \pm 0.93]\) and high dose \([92 \pm 0.65]\) of extract brought extremely significant increase in SOD levels when compared with diseased control \([21 \pm 0.79]\).

Streptozotocin brought extremely significant decrease in CAT level \([182 \pm 0.86]\) when compared with normal animals \([501 \pm 0.21]\). Treatment with low dose \([457 \pm 0.78]\) and high dose \([469 \pm 0.32]\) of extract brought extremely significant increase in CAT levels when compared with diseased control \([182 \pm 0.86]\). Streptozotocin brought extremely significant increase in GSH level \([18 \pm 0.39]\) and high dose \([58 \pm 0.61]\) of extract brought extremely significant increase in GSH levels when compared with diseased control \([18 \pm 0.48]\). Streptozotocin brought extremely significant increase \([88 \pm 0.38]\) in MDA level when compared with normal animals \([19 \pm 0.13]\). Treatment with low dose \([31 \pm 0.21]\) and high dose \([26 \pm 0.82]\) of extract brought extremely significant decrease in MDA levels when compared with diseased control \([88 \pm 0.38]\).

Streptozotocin brought extremely significant increase \([407 \pm 0.54]\) in TC level when compared with normal animals \([117 \pm 0.46]\). Treatment with low dose \([154 \pm 0.67]\) and high dose \([142 \pm 0.52]\) of extract brought extremely significant decrease in TC levels when compared with diseased control \([407 \pm 0.54]\). Streptozotocin brought extremely significant increase \([363 \pm 0.53]\) in TG level when compared with normal animals \([91 \pm 0.69]\). Treatment with low dose \([125 \pm 0.32]\) and high dose \([114 \pm 0.52]\) of extract brought extremely significant decrease in TG levels when compared with diseased control \([363 \pm 0.53]\). Streptozotocin brought extremely significant decrease \([12 \pm 0.34]\) in HDL level when compared with normal animals \([55 \pm 0.17]\). Treatment with low dose \([42 \pm 0.29]\) and high dose \([47 \pm 0.45]\) of extract brought extremely significant increase in HDL levels when compared with diseased control \([12 \pm 0.34]\). Streptozotocin brought extremely significant increase \([159 \pm 0.81]\) in LDL level when compared with normal animals \([49 \pm 0.73]\). Treatment with low dose \([75 \pm 0.65]\) and high dose \([63 \pm 0.22]\) of extract brought extremely significant decrease in LDL levels when compared with diseased control \([159 \pm 0.81]\).

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<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diseased</th>
<th>Low Dose</th>
<th>High Dose</th>
<th>Standard</th>
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<tbody>
<tr>
<td><strong>Body Weight [g]</strong></td>
<td>220 ± 0.37</td>
<td>131 ± 0.62***</td>
<td>202 ± 0.71###</td>
<td>206 ± 0.92###</td>
<td>213 ± 0.53***</td>
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<tr>
<td><strong>Blood Glucose [mg/dl]</strong></td>
<td>110 ± 0.47</td>
<td>331 ± 0.46###</td>
<td>139 ± 0.61###</td>
<td>131 ± 0.51###</td>
<td>126 ± 0.78**</td>
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<tr>
<td><strong>SOD [U/ml]</strong></td>
<td>103 ± 0.74</td>
<td>21 ± 0.79***</td>
<td>90 ± 0.93###</td>
<td>92 ± 0.65###</td>
<td>98 ± 0.41###</td>
</tr>
<tr>
<td><strong>CAT [U/ml]</strong></td>
<td>501 ± 0.21</td>
<td>182 ± 0.86###</td>
<td>457 ± 0.78###</td>
<td>469 ± 0.32###</td>
<td>485 ± 0.39###</td>
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<tr>
<td><strong>GSH [μmol/ml]</strong></td>
<td>67 ± 0.54</td>
<td>18 ± 0.48###</td>
<td>53 ± 0.39###</td>
<td>58 ± 0.61###</td>
<td>61 ± 0.42###</td>
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<tr>
<td><strong>MDA [μmol/L]</strong></td>
<td>19 ± 0.13</td>
<td>88 ± 0.38###</td>
<td>31 ± 0.21###</td>
<td>26 ± 0.82###</td>
<td>22 ± 0.53###</td>
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<tr>
<td><strong>TC [mg/dl]</strong></td>
<td>117 ± 0.46</td>
<td>407 ± 0.54###</td>
<td>154 ± 0.67###</td>
<td>142 ± 0.52###</td>
<td>131 ± 0.72###</td>
</tr>
<tr>
<td><strong>TG [mg/dl]</strong></td>
<td>91 ± 0.69</td>
<td>363 ± 0.53###</td>
<td>125 ± 0.32###</td>
<td>114 ± 0.52###</td>
<td>104 ± 0.18###</td>
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<tr>
<td><strong>HDL [mg/dl]</strong></td>
<td>55 ± 0.17</td>
<td>12 ± 0.34###</td>
<td>42 ± 0.29###</td>
<td>47 ± 0.45###</td>
<td>51 ± 0.24###</td>
</tr>
<tr>
<td><strong>LDL [mg/dl]</strong></td>
<td>49 ± 0.73</td>
<td>159 ± 0.81###</td>
<td>75 ± 0.65###</td>
<td>63 ± 0.22###</td>
<td>57 ± 0.36###</td>
</tr>
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</table>

All values are expressed as MEAN ± SEM. N = 6. One-way ANOVA, followed by Bonferroni’s test (all) used. ***p < 0.001= diseased control group when compared with normal group. ###p < 0.001= low, high dose and standard treated groups when compared with diseased control group.

Insulin-dependent diabetes mellitus is a condition brought on by streptozotocin's inhibition of insulin production. Its unique chemical characteristics, specifically its alkylating potency, are responsible for both effects.[12] The DNA alkylating activity of streptozotocin's methylnitrosourea moiety, particularly at the O6 position of guanine, is generally believed to be responsible for the drug's toxicity. As a result of damage brought on by the transfer of the methyl group from streptozotocin to the DNA molecule, the DNA fragments as a result of a predetermined series of events. Glycosylation of proteins might also be harmful. Poly(ADP-ribose) polymerase (PARP) is overstimulated in an effort to repair DNA. As a result, cellular NAD+ and ATP reserves are reduced. Beta cell necrosis is the end outcome of the depletion of cellular energy reserves. Beta cell death is eventually caused by DNA methylation.[12]
Methanolic extract of fruit of Phyllanthus reticulatus may contain alkaloids, flavonoids, terpenoids, polyphenolic compounds, lignans. Phyllanthus reticulatus fruit extract showed antioxidant activity. It might possess free radical scavenging activity.\(^{[13]}\)

4. Conclusion
Phyllanthus reticulatus fruit extract demonstrated anti diabetic activity in the present study. Pharmacological estimations supported the above statement. However, further studies needed, to work out the exact mechanism, by which, the extract, could be exerting its effects.

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References: