ANTI-DIABETIC EFFECT OF THE ETHANOLIC EXTRACT OF DRIED FRUITS OF *ADANSONIA DIGITATA* LINN.

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ABSTRACT

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products obtained from plant, animal and minerals have been the basis of the treatment of human disease. Today it is estimated that about 80% of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. The World Health Organization has defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. The plant based raw materials are safe, preventive, curative and are particularly useful in achieving the goal of “Health to All” in a cost effective manner. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. Herbal medicines are currently in demand and their popularity is increasing day by day. Particularly, the herbal medicines are getting more importance in the treatment of diabetes and others ailments because of the hazardous adverse effect of the current therapy used to treat those ailments using synthetic drugs. Herbal medicine is free from side effects and less costly when compared to the synthetic hypoglycemic agents. The present study will help the industry to produce herbal drug with less side effect, less costly, thus affordable and more effective in the treatment of diabetes mellitus. In vivo model was utilized to test the anti diabetic activity.

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Article Received on 20 March. 2017,
Revised on 10 April 2017,
Accepted on 30 April 2017
DOI: 10.20959/wjpps20175-9217

INTRODUCTION[1-9]
Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and defective metabolism of glucose and lipids. Diabetes was estimated to affect 300 million people by 2025. Diabetes is not a single disease rather it is a heterogeneous group of syndromes characterized by an elevation of blood glucose caused by relative or absolute deficiency of insulin. Diabetes can be divided into two main groups based on their requirements of insulin: insulin dependent diabetes mellitus (Type 1) and non-insulin dependent diabetes mellitus (Type 2). However, other types of diabetes have also been identified. Maturity Onset Diabetes of the Young (MODY) is now classified as Type 3 and gestational diabetes classified as Type 4. NIDDM type 2 diabetes account for about 90 percent of diabetic cases. Insulin resistance and β-cell dysfunction are the metabolic abnormalities in the type 2 diabetes. Glycemic control is one of the targets for managing diabetes mellitus. Studies have confirmed that for the type 2 diabetes, effective control of blood glucose substantially decrease the risk of developing diabetic complications.

Orthodox treatment of diabetes mellitus includes a modification of life style, such as diet and exercise and the use of insulin and/or oral hypoglycemic drugs. These pharmacologic agents target increased insulin secretion, decreased hepatic glucose production and increased sensitivity to insulin. Management of this disease with insulin and/or oral hypoglycemic agents have certain drawbacks. For insulin such drawbacks include ineffectiveness on oral administration, short shelf life, requirement of constant refrigeration and in the event of excess dosage-fatal hypoglycemia. The use of oral hypoglycemic drugs like sulfonylurea's and biguanides is also associated with side effects such as propensity to gain weight. Adansonia digitata L. (Bombacaceae) An African plant known as baobab tree. Leaves, bark and fruits of this plant are traditionally employed in several African regions as food stuffs and for medicinal purposes, and for that reason baobab is also named “the small pharmacy or chemist tree”. The native African population commonly used, baobab fruit as famine food to prepared, decoctions, sauces and natural refreshing drink due to it nutritional properties.

The pulp is therapeutically employed as analgesic, anti-diarrheal and for treatment of smallpox and measles. The present study was designed to test the anti-diabetic effect of ethanolic extract of Adansonia digitata L. fruits on streptozotocin- induced diabetes on rats.
MATERIALS AND METHODS

Plant Collection, Identification And Processing
The fresh fruits of *Adansonia digitata* L., was collected from the Madras Medical College Men's Hostel, Chennai, Tamilnadu. The plant material was authenticated by Botanist Prof. P. Jayaraman Ph.D., Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Tambaram, Chennai, Tamilnadu. The fruits were shade dried, coarsely powdered and used for further studies. The dried coarsely powdered sample of *Adansonia digitata* L., fruits (500gm) was extracted with Ethanol in Soxhlet apparatus (60-70°C). The extract was concentrated using rotary vacuum evaporator.\(^\text{[10]}\)

Phytochemical Analysis\(^\text{[11]}\)
The ethanol extract of *Adansonia digitata* L. the was subjected to phytochemical screening tests to detect the presence of carbohydrates, anthraquinones, flavonoids, tannins, alkaloids, saponins, glycosides, sterols and triterpenes.

Acute Toxicity Study\(^\text{[12]}\)
Literature review showed that the acute toxicity study on fruit extracts of *Adansonia digitata* Linn., was performed and the extract did not produced toxicity till the dose level of 5000 mg/kg. Hence, a starting dose level of 200 mg/kg of fruits of *Adansonia digitata* Linn., was used. After oral administration, animals were observed at an hourly basis for the first 4 hours and periodically for 24 hours to assess the general behaviour and 72 hours for any toxic symptoms and mortality of the animal for 28 days.

Ethical Committee Approval
The protocol for conducting the *in vivo* study in wistar albino rats was approved by the Institutional Animal Ethical Committee (IAEC) which is certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Approval no: IAEC/MMC/07/2016.

Experimental Design For Streptozotocin Induced Hyperglycemic Studies\(^\text{[13,14,15]}\)
Animals were randomly divided into 5 groups of rats (n=6)
The animals received the following treatments,
Table No: 1 Experimental design for anti-diabetic activity

<table>
<thead>
<tr>
<th>S.NO</th>
<th>GROUP</th>
<th>NAME OF THE DRUG</th>
<th>DOSE</th>
<th>NO. OF ANIMALS</th>
<th>DURATION OF DOSAGE (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group-1</td>
<td>Normal control</td>
<td>Saline</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Group-2</td>
<td>Diabetic control (0.9% v/v saline)</td>
<td>2ml p.o.</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>Group-3</td>
<td>Glibenclamide</td>
<td>4mg/kg p.o</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>Group-4</td>
<td>Extract low dose</td>
<td>200mg/kg p.o</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Group-5</td>
<td>Extract high dose</td>
<td>400mg/kg p.o</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TOTAL</td>
<td>30</td>
<td>28</td>
<td></td>
</tr>
</tbody>
</table>

The experimental animals were fasted for 18 hours and the blood glucose level (BGL) was monitored using a glucometer after streptozotocin injection. Blood samples was collected by tail clipping method. Rats with blood glucose level of greater than 250 mg/dl were considered diabetic and selected for the study (WHO, 1985). Rats were randomly divided into 5 groups of 6 rats per group for screening.

Streptozotocin monohydrate 45 mg/kg body weight was dissolved in 0.9% v/v cold normal saline and injected intraperitoneally to 18 hours fasted rats (24 no’s,) group II-IV in order to induce hyperglycemia in experimental wistar rats (130-180g body weight (b/w) and the six control rats (group-I) received equal volume of 0.9% v/v cold normal saline injected intraperitoneally.

Collection Of Blood And Organs
The treatment was carried up to 28 days and on 1st, 7th, 14th and 21st days 0.5 ml of blood was collected from lateral tail vein using lance or butterfly needle and blood glucose level was checked by using a Glucometer.

After 28 days the blood was collected and used to determine hematological parameters. The test animals were anesthetized with ketamine hydrochloride at the dose of 10 mg/kg and sacrificed. Pancreas was isolated and used for histopathological studies.

Histopathological Study[16]
For histological examinations, small pieces of pancreas were fixed in Bouin's Solution for 24h dehydrated through graded concentration of ethanol, embedded in Paraffin wax, sectioned at 5µm thicknesses and stained with Mayer's haematoxylin and Eosin and observed under light microscope.
Statistical Analysis
Results were expressed as Mean ± S.E.M. The data was analyzed using One Way of Variable (ANOVA) followed by Dennett’s test. P-value <0.05 considered as significant.

RESULTS AND DISCUSSION
Extract Yield
The plant gave a yield of 5.27 % of dark reddish brown dried crude ethanolic extract.

Phytochemical Test
The phytochemical test was performed and the ethanol extract of dried fruits of *Adansonia digitata* L. showed the presence of alkaloids, glycosides, steroids, flavonoids, saponins, carbohydrates, gums and mucilage.

Streptozotocin Induced Diabetes Mellitus In Rats
Table No: 2 Effects of Ethanolic extract of *Adansonia digitata* Linn., on blood glucose level in streptozotocin induced diabetic rats (mg/dl)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 7</th>
<th>DAY 14</th>
<th>DAY 21</th>
<th>DAY 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I (Normal)</td>
<td>103 ± 1.4</td>
<td>96 ± 1.3</td>
<td>94 ± 1.5</td>
<td>99 ± 1.3</td>
<td>101 ± 1.1</td>
<td>95 ± 1.9</td>
</tr>
<tr>
<td>GROUP II (Diabetic)</td>
<td>108 ± 2.3</td>
<td>410 ± 1.9</td>
<td>356 ± 1.5</td>
<td>347 ± 1.6</td>
<td>304 ± 2.6</td>
<td>296 ± 2.2</td>
</tr>
<tr>
<td>GROUP III (Standard)</td>
<td>111 ± 1.6</td>
<td>397 ± 3.4</td>
<td>96 ± 2.8</td>
<td>89 ± 1.5</td>
<td>84 ± 2.2</td>
<td>79 ± 3.3</td>
</tr>
<tr>
<td>GROUP IV (Low dose)</td>
<td>105 ± 2.2</td>
<td>418 ± 2.0</td>
<td>111 ± 1.5</td>
<td>107 ± 4.2</td>
<td>106 ± 1.7</td>
<td>97 ± 1.6</td>
</tr>
<tr>
<td>GROUP V (High dose)</td>
<td>115 ± 1.5</td>
<td>405 ± 3.3</td>
<td>142 ± 1.1</td>
<td>130 ± 2.7</td>
<td>126 ± 1.2</td>
<td>118 ± 2.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD; n = 6; P < 0.05 compared to diabetic control

Fig no: 1 Graphical representation of blood glucose level in study groups (mg/dl)
Table No: 3 Effects of Ethanolic extract of *Adansonia digitata* Linn., on body weight in streptozotocin induced diabetic rats (mg/dl)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 7</th>
<th>DAY 14</th>
<th>DAY 21</th>
<th>DAY 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP I (Normal)</td>
<td>135 ± 5.68</td>
<td>138 ± 9.43</td>
<td>143 ± 3.43</td>
<td>147 ± 3.47</td>
<td>133 ± 5.32</td>
<td>146 ± 2.22</td>
</tr>
<tr>
<td>GROUP II (Diabetic)</td>
<td>149 ± 5.75</td>
<td>141 ± 7.22</td>
<td>129 ± 6.32</td>
<td>103 ± 4.33</td>
<td>97 ± 3.55</td>
<td>93 ± 1.55</td>
</tr>
<tr>
<td>GROUP III (Standard)</td>
<td>157 ± 7.06</td>
<td>154 ± 6.48</td>
<td>137 ± 6.75</td>
<td>144 ± 5.67</td>
<td>152 ± 9.76</td>
<td>166 ± 6.78</td>
</tr>
<tr>
<td>GROUP IV (Low dose)</td>
<td>149 ± 7.75</td>
<td>145 ± 8.67</td>
<td>105 ± 6.97</td>
<td>123 ± 7.55</td>
<td>145 ± 5.54</td>
<td>149 ± 2.37</td>
</tr>
<tr>
<td>GROUP V (High dose)</td>
<td>151 ± 1.25</td>
<td>144 ± 7.77</td>
<td>101 ± 3.21</td>
<td>119 ± 3.34</td>
<td>122 ± 7.64</td>
<td>136 ± 3.54</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD; n = 6; P < 0.05 compared to diabetic control.

Fig no: 2 Graphical representation of body weight level in study groups (grams)

HISTOPATHOLOGICAL EXAMINATION OF RAT PANCREAS

Fig no: 3 Normal control group  
Fig no: 4 Diabetic control group
Fig no: 5 Standard (Glibenclamide 4mg/kg)

Fig no: 6 Adansonia digitata Extract (200 mg/kg)

Fig no: 7 Adansonia digitata Extract (400 mg/kg)

Fig no: 38 Presence of normal pancreatic islet cells.

Fig no: 39 Reduction in the size of islets, damaged β-cell population and extensive necrotic changes followed by fibrosis and atrophy.

Fig no: 40 Restored necrotic and fibrotic changes. Increased number and size of the islets.

Fig no: 41&42 Absence of necrosis and fibrotic changes. Increased number and size of the islets and presence of normal pancreatic islet cells.

CONCLUSION
The Anti-diabetic activity of extract was accessed by the method of streptozotocin induced diabetes in rats. In this method, parameters like blood glucose level and body weight was evaluated. The blood glucose level of the standard group and extract treated groups significantly decreased when compared to disease control group and the body weight of the standard group and extract treated groups significantly increased when compared to disease control group. Histopathological study results showed that the cells in the diabetic control group were reduced in size, damaged β-cell population and extensive necrotic changes,
followed by fibrosis and atrophy. While in the group that received the test dose showed, the absence of necrosis, fibrotic changes, increased number and size of the islets and presence of normal pancreatic cells. These were in the levels comparable with the ones that were administered the standard drug glibenclamide. In this study, the experiment evidence obtained indicates that ethanolic extract of *Adansonia digitata* Linn., fruits possess the anti-diabetic properties which suggests the presence of biologically active components which may be worth further investigation and elucidation.

REFERENCES


