Evaluation of Antidiabetic Activity of a Herbal Formulation

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Abstract

Since diabetes has become prevalent in huge number of people worldwide it was aimed to prepare a formulation containing herbal drugs and scientifically evaluate it for antidiabetic activity. A polyherbal formulation was prepared containing herbs reported to have good antidiabetic activity and widely used traditionally in India by the folklore for the treatment of diabetes since ages. They are used in Ayurveda and other Indian traditional system of medicine.

Herbal formulation was prepared with the mixture of alcoholic extract of Tribulus terrestris fruits, Boerhavia diffusa roots and Azadirachta indica leaves. Its organoleptic and physicochemical parameters were evaluated. Antidiabetic activity using 100 mg/kg and 200 mg/kg of formulation was studied in streptozotocin induced diabetic albino Wistar rats. Glibenclamide was used as standard. Studies were done for 28 days. Change in body weight and blood glucose level was estimated. In the diabetic rats treated with herbal formulation, the weight of pancreas increased in a dose dependent manner. The blood glucose level decreased significantly P < 0.001 in the standard and formulation treated groups when compared to diabetic control group. Histopathology of pancreas of herbal formulation treated animals showed protective and regenerative effect of the pancreatic cells. The dose of 200 mg/kg had better effect than the standard drug glibenclamide. The herbal formulation is a potent antidiabetic and safe for use.

Keywords: Antidiabetic activity; Azadirachta indica; Boerhavia diffusa; Tribulus terrestris

Introduction

Diabetes is due to insufficient insulin production or its ineffectiveness. It is a global problem [1]. Diabetes mellitus is a chronic complication of derangement of protein, carbohydrate and fat metabolism arising due to defective insulin secretion or action or both [2,3]. This disorder of metabolism results in hyperglycemia and glycosuria. The blood sugar level is high over a prolonged period. It develops by regular consumption of sugar sweetened beverages [4] and sedentary lifestyle [5]. Diabetes mellitus (DM) is considered to be one of the most serious endocrine syndromes. In many countries it is traditional to use plants to control diabetes [6,7].

Many plants have been used traditionally in Ayurveda and reported to have good antidiabetic activity [8-11]. Antidiabetic activity has been studied in polyherbal formulation [12]. Rajan, et al. have reported that the maximum phytotherapeutic efficacy can be achieved by the combination of two or more plants rather than one [13]. Many polyherbal formulations have been developed and evaluated for their activities [14].

Therefore a herbal formulation based on the medicinal values of herbs was prepared. It contained Tribulus terrestris fruits, Boerhavia diffusa roots and Azadirachta indica leaves. Tribulus terrestris L. (Zygophyllaceae) commonly known as Gokhru has analgesic, anti-inflammatory and diuretic activity [15]. It also has significant hypoglycemic and hypolipidemic effect [16]. Boerhavia diffusa Linn. (Nyctaginaceae) is a cardiotonic [17] having hepatoprotective [18], anti-inflammatory, antibacterial, and antidiabetic [19-21] activity. Azadirachta indica A. Juss (Meliaceae) have been used by folklore for a number of ailments. It has been reported to have antimutagenic [22], antidiabetic [23,24] and anti-hyperlipaemic activity [25]. As these plants showed good antidiabetic activity they were used to prepare a herbal formulation having additive and/or synergistic effect.

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Materials and Methods

Plant material

Preparation of Herbal Formulation (TAB)

The formulation was prepared by trituration method [13]. The coarse powder of all the three drugs was macerated individually with alcohol, filtered and concentrated to get the corresponding extracts. These extracts were taken in equal quantity (1:1:1) and mixed well with Tween 80 by trituration. A uniformly dispersed formulation was prepared.

Animals

Albino wistar rats weighing between 175 ± 25 gm were used for the study. They were kept at a of temperature 22 ± 2°C. All animals were given standard diet (Golden feed N. Delhi, India) and water ad libitum, with 12:12 hour light:dark cycle. The protocol was approved by the Institutional Animal Ethics Committee (1413/a/11/CPCSEA) as per CPCSEA guidelines. The protocol approval no. is PBRI/IAEC/13-14/PN-352.

Acute toxicity

OECD guidelines were followed for the study of acute toxicity. The rats were given dose orally till 2000mg/kg. Any change in their behavior or mortality was observed.

Assessment of Antidiabetic activity [26]

For the study the rats were taken randomly. All the animals were administered their respective doses orally, except that 60 mg/kg of streptozotocin (STZ) was given intraperitoneally to induce diabetes in Group II, III, IV and V. Study was done for 28 days.

The animals were divided into six groups each containing six rats:

- Group I: assigned as vehicle control was given only normal saline daily.
- Group II: served as negative/diabetic control was administered normal saline daily.
- Group III: served as standard was given standard drug glibenclamide 600 µg/kg per day
- Group IV: received herbal formulation TAB 100 mg/kg per day
- Group V: received herbal formulation TAB 200 mg/kg per day

The animals of Group II to V were allowed to drink 5% glucose solution overnight to overcome STZ induced hyperglycaemia. On 3rd day of STZ injection their blood glucose was measured. The animals having above 200 mg/dl were considered as diabetic. On 4th day the respective dosing was started considering it as 1st day of treatment and was continued till 28 days. Body weight and blood glucose level was observed on 0, 7, 14, 21 and 28 day of post treatment (Table 1 and 2; Figure 1 and 2). On 28th day the rats were sacrificed under mild ether anesthesia. The pancreas were transferred to 10% formalin solution and immediately processed by the paraffin technique. The sections were stained by haematoxylin and eosin (H & E) for histological examination (Figure 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>0 Day</th>
<th>7 Day</th>
<th>14 Day</th>
<th>21 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle (Saline) (5ml/kg)</td>
<td>194.5 ± 19.013</td>
<td>197.3 ± 19.065</td>
<td>198.5 ± 20.167</td>
<td>199.5 ± 20.599</td>
<td>199.8 ± 20.605</td>
</tr>
<tr>
<td>II</td>
<td>Vehicle + STZ (60 mg/kg)</td>
<td>186.3 ± 19.511</td>
<td>180.2 ± 17.804</td>
<td>170.8 ± 19.793</td>
<td>169.7 ± 18.790</td>
<td>167.5 ± 17.398</td>
</tr>
<tr>
<td>III</td>
<td>STZ + Glibenclamide (600 µg/kg)</td>
<td>176.0 ± 17.498</td>
<td>184.7 ± 19.387</td>
<td>190.3 ± 22.196</td>
<td>192.0 ± 21.909</td>
<td>194.7 ± 19.997</td>
</tr>
<tr>
<td>IV</td>
<td>STZ + TAB (100 mg/kg)</td>
<td>147.5 ± 12.755*</td>
<td>150.8 ± 13.045*</td>
<td>152.3 ± 13.049*</td>
<td>154.8 ± 12.416*</td>
<td>156.5 ± 11.962*</td>
</tr>
<tr>
<td>V</td>
<td>STZ + TAB (200 mg/kg)</td>
<td>204.7 ± 25.547*</td>
<td>208.0 ± 24.487*</td>
<td>211.8 ± 25.214*</td>
<td>214.7 ± 24.229*</td>
<td>216.7 ± 25.240*</td>
</tr>
</tbody>
</table>

**Table 1:** Effect of herbal formulation TAB on Body weight. Values are expressed as MEAN ± SD at n = 6

*P < 0.001 compared to the Streptozotocin (STZ), NS – non significant

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<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood Glucose Level (mg/dl)</th>
<th>0 Day</th>
<th>7 Day</th>
<th>14 Day</th>
<th>21 Day</th>
<th>28 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Vehicle (Saline) (5 ml/kg)</td>
<td></td>
<td>85.7 ± 8.519</td>
<td>87.4 ± 7.830</td>
<td>88.5 ± 7.968</td>
<td>89.3 ± 7.460</td>
<td>89.5 ± 9.269</td>
</tr>
<tr>
<td>II</td>
<td>Vehicle + STZ (60 mg/kg)</td>
<td></td>
<td>262.2 ± 13.674</td>
<td>267.3 ± 14.473</td>
<td>271.8 ± 14.414</td>
<td>275.5 ± 13.308</td>
<td>275.7 ± 15.148</td>
</tr>
<tr>
<td>III</td>
<td>STZ + Glibenclamide (600 µg/kg)</td>
<td></td>
<td>256.0 ± 10.020 NS</td>
<td>170.0 ± 10.315 *</td>
<td>132.5 ± 7.092 *</td>
<td>108.9 ± 8.954 *</td>
<td>89.2 ± 7.083 *</td>
</tr>
<tr>
<td>IV</td>
<td>STZ + TAB (100 mg/kg)</td>
<td></td>
<td>207.5 ± 12.046 *</td>
<td>95.8 ± 11.618 *</td>
<td>92.2 ± 3.710 *</td>
<td>88.3 ± 46.449 *</td>
<td>86.2 ± 6.853 *</td>
</tr>
<tr>
<td>V</td>
<td>STZ + TAB (200 mg/kg)</td>
<td></td>
<td>213.3 ± 17.259 *</td>
<td>111.3 ± 18.162 *</td>
<td>96.5 ± 3.937 *</td>
<td>85.2 ± 11.669 *</td>
<td>80.8 ± 5.879 *</td>
</tr>
</tbody>
</table>

Table 2: Effect of herbal formulation TAB on Blood Glucose Level (mg/dl).
Values are expressed as MEAN ± SD at n = 6

*P < 0.001 compared to the Streptozotocin (STZ), NS – non significant

Figure 1: Effect of herbal formulation TAB on Body weight.

Figure 2: Effect of herbal formulation TAB on Blood Glucose Level (mg/dl).

Figure 3: Histology of rat pancreas for antidiabetic activity of herbal formulation TAB.
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Statistical analysis
All data were analyzed by One way ANOVA followed by Bonferroni test. P < 0.001 was considered as level of significance. All data are presented in Mean ± SD.

Results
In acute toxicity studies none of the animal died till a dose of 2000 mg/kg. No abnormal behavior was found. Hence LD50 was more than 2 gm/kg. In antidiabetic study the body weight of rats was found to decrease in the STZ diabetic control group whereas it increased in standard glibenclamide and herbal formulation groups. It increased significantly P < 0.001 in TAB 200 mg/kg animals on 14th day onwards as compared to the diabetic control group. The results of TAB 200 mg/kg were better than the standard drug. The blood glucose level decreased significantly P < 0.001 in the standard and TAB groups when compared to diabetic control group. In TAB groups the lowering of glucose was very fast in the first week reaching the normal range, than it gradually slowed down. Both the doses of TAB showed very good antidiabetic activity.

Diabetes has become a major health problem in most of the countries. Combination of herbs have been extensively used from ancient time and shown potent antidiabetic activities without toxicity. Therefore a polyherbal formulation was prepared. Ponnusamy., et al have reported that T. terrestris has strong inhibitory action on human pancreatic α- amylase thereby contributing in antidiabetic activity [27]. Since STZ has selective pancreatic islet beta cell cytotoxicity it is used to induce type I diabetes in rat model [28]. Streptozotocin enters the β cell causing alkylation of DNA resulting in necrosis [29]. Lack of insulin leads to inactivation of the glycogen synthetase systems [30]. The possible mechanism of lowering blood glucose level by herbal formulation TAB may be by inhibiting the pancreatic enzyme resulting in an increase in the pancreatic secretion of insulin or its release from the bound form.

Discussion
Diabetes has become a major health problem in most of the countries. Combination of herbs have been extensively used from ancient time and shown potent antidiabetic activities without toxicity. Therefore a polyherbal formulation was prepared. Ponnusamy., et al have reported that T. terrestris has strong inhibitory action on human pancreatic α- amylase thereby contributing in antidiabetic activity [27]. Since STZ has selective pancreatic islet beta cell cytotoxicity it is used to induce type I diabetes in rat model [28]. Streptozotocin enters the β cell causing alkylation of DNA resulting in necrosis [29]. Lack of insulin leads to inactivation of the glycogen synthetase systems [30]. The possible mechanism of lowering blood glucose level by herbal formulation TAB may be by inhibiting the pancreatic enzyme resulting in an increase in the pancreatic secretion of insulin or its release from the bound form.

Conclusion
The results show that the herbal formulation TAB is safe and able to control the increased blood glucose level. Its potent antidiabetic activity supports its traditional use. Hence it can be used as an effective oral antidiabetic herbal medicine.

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Conflict of Interest
Declared none.

Bibliography


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