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A new antidiabetic polyherbal formulation for diabetes

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ABSTRACT

Background: Synergism is a well-known concept in Herbal therapies. Polyherbal aqueous extract (PH-AE) containing Gymnema sylvestre (GS), Momordica charantia, Syzygium cumini, and Mangifera indica was evaluated for Antidiabetic activity. Objective: In our continuous research, an attempt has been made to explore GS aqueous extract (GS-AE) and its Polyherbal composite for diabetes to achieve greater efficacy. Methods: The antidiabetic activity was assessed in Streptozotocin induced diabetic rats and histopathological changes in Pancreas were documented after screening acute toxicity of both extracts. Results: Chromatographic separation and identification of GS-AE revealed the presence of Saponins and glycosides, which have also been confirmed in PH-AE by Phytochemical tests. Though GS-AE showed good control over blood sugar, after 21 days GS-AE and Glibenclamide treated groups still had 56.25% and 41.60% sugar levels, respectively, in hyperglycemic rats whereas Polyherbal extracts exhibited highly significant (P ≤ 0.001) antidiabetic activity when compared to Glibenclamide by leaving only 6% increased residual sugar level in comparison to control. The activity was also substantiated by normalization of histopathological architecture of pancreas. Conclusion: Our results indicate that Polyherbal extract shows synergism among its components, which could emerge as a novel potent antidiabetic formulation to combat diabetes through clinical trials.

Keywords: Antidiabetic, Glibenclamide, Gymnema sylvestre, Phytoconstituents, Polyherbal

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder, resulting in insulin deficiency-characterized by hyperglycemia. The metabolic abnormalities lead to symptoms of Polyurea (frequent urination), Polydipsia (excessive thirst), polyphagia (excessive hunger), etc. Long-term untreated DM might lead to gangrene, retinopathy, myocardial infarction, and sometimes high blood pressure.¹²

DM is categorized in DM Type-1 and DM Type-2. Oral Hypoglycemic agents are used in the treatment of patient who have Type-2 diabetes and cannot be managed by diet alone.¹¹ Patient who have developed diabetes after the age of 40 and have diabetes for <5 years respond well to the oral hypoglycemic agent, while those with long standing disease may require a combination of oral hypoglycemic agent with or without insulin to control their hyperglycemia.¹³

For the treatment of chronic disorders, that is, diabetes, traditional medicines have suggested the use of herbal therapy since time immemorial. Ayurveda has believed in polyherbalism and synergistic use of herbs as evidenced in treatises of Ayurveda containing Ayurvedic formulations, for example, Charak Samhita and Bhaishjya Ratnawali.¹⁴

Gymnema sylvestre (GS) belongs to the family Asclepiadaceae, and local to the tropical woodlands of India, where it has been utilized as a naturopathic treatment for diabetes for almost two centuries. In conventional drug, it is very much considered as a solution for Diabetes, stomachic and anti-diarrheal agent. The plant is prevalently known as “Gurmar” for its property of destroying the flavor of sweetness. The primary bioactive constituents of GS are mostly saponins known as gymnemic acids which have been accounted for as antidiabetic, and anti-cholesterol agent in the in vitro studies.⁵⁻¹⁰

Momordica charantia (MC), also known as bitter melon, karela, balsam pear or bitter gourd belongs to Cucurbitaceae family. It is accepted remedy for the management of diabetes
in Asia, South America, India, the Caribbean, and East Africa.\textsuperscript{11,12} Its fruit has a characteristic bitter taste. Several studies have established the anti-diabetic effects of MC. It also has significant hypolipidemic activity; hence, it has a potential to be employed as an adjuvant to delay the diabetic complications simultaneously with allopathic treatment.\textsuperscript{13-16}

*Syzygium cumini* (L.) Skeels (jambolan) belonging to Myrtaceae family, is an important plant for herbal intervention for diabetes.\textsuperscript{8,12} Its seeds are reported to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar.\textsuperscript{17-20}

For the treatment of lifestyle disorders, that is, diabetes, the use of dietary therapy recommends hope for a safe and efficient medication. It has also been established by epidemiological studies that have shown an inverse relation between the risk of diabetes and the consumption of polyphenolic rich diet. Several studies have shown that *Mangifera indica* (MI) kernel flour is a rich source of pharmacologically important flavonoids (catechin, rutin, quercitrin, quercetin, and kaempferol) and phenolic acids (gallic acid, caffeic, chlorogenic, and ellagic acid).\textsuperscript{21-25}

Polyherbal extract can improve the pharmacological activity and decreases the effective dosage of single herb which can prevent the occurrence of adverse effects. Combined extract of plants rather than a single ingredient might prove to be more effective however exploring an effective composition either single or in combination against diabetes is still tricky.\textsuperscript{11,12}

Based on above observation, the present study was planned to compare the antidiabetic efficacy of GS leaves and its polyherbal composite extracts in a Streptozotocin (STZ) induced diabetes rat model using Glibenclamide as standard treatment.

**MATERIALS AND METHODS**

**Chemicals and Herbs**

Glibenclamide and all the other chemicals and solvents used were of analytical grade. Herbal materials used were GS leaves, MC fruits, SC seeds, and MI seed kernel.

**Collection and Authentication of Herbs**

All the herbal materials were collected from M/S Global Herbs, New Delhi. The authentication and identification was done by Dr. Sunita Garg, the Emeritus Scientist, CSIR-NISCAIR, Delhi. Other than leaves, all other herbs were coarsely ground. The powdered material was sieved over \# sieve. The retained material was used for extraction purpose.

**Extraction Procedure**

GS leaves (1 kg) and Polyherbal blend (250 g each of GS, MC, SC, and MI) were twice extracted with water (2 l) for 72 h with frequent agitation. The extract thus obtained was concentrated by distilling off the solvent under reduced pressure using Rota vacuum Evaporator (Rotavapor R-300, Buchi, Germany) to yield GS aqueous extract (GS-AE) and polyherbal aqueous extract (PH-AE), respectively.\textsuperscript{26}

Both the extracts were subjected to Phytochemical screening.\textsuperscript{26} Column Chromatographic separation of GS-AE was performed using Silica gel (60–120 \( \mu \)m) and identification of the components was carried out by thin-layer chromatography (TLC) using Chloroform-Methanol-Formic acid-Water (10:4:1:0.95) as mobile phase and water as visualizing agent.

**Animals**

Wistar rats (150–200 gm; 8–11 weeks old) were obtained from AIIMS, New Delhi (India). The animals were housed in the Animal house, I.TS College of Pharmacy, Ghaziabad, India in polycarbonate cages, in a room maintained under controlled condition (22 ± 2°C, relative humidity 60–70%) and provided with food and water ad libitum. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Reg. No.:1044/PO/Re/S/07/CPCSEA and Protocol No. ITS/03/IAEC/2018) and the care of laboratory animals were taken as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India. Before the experimentation, the animals were deprived of food for 24 h but were allowed free access to water throughout. The studies were carried out using six animals in each group.

**Acute Toxicity Study**

The acute toxicity was performed according to the Organization for Economic Cooperation and Development (423, 2001) guidelines.\textsuperscript{26} Extracts were given orally to rats at the graded dose of 1000, 2000, and 4000 mg/kg body weight. After dosing, the animals were observed continuously for first 4 h for behavioral changes and for mortality and then daily up to 14 days.\textsuperscript{27}

**Antidiabetic Activity: STZ Induced Diabetes Model**

**Induction of diabetes in animals**

STZ was freshly prepared using 0.1M citrate buffer, pH 4.5. The male Wistar rats (175 ± 35.36), fed with standard diet (150–220 g) were injected with STZ (60 mg/kg, i.p.). After 72 h of STZ administration, the blood glucose levels were measured using Accu-Chek Active Glucose meter and the rats showing blood glucose level >250 mg/dl were considered to be diabetic and were used in the study. Treatment with GS-AE and PH-AE started after the last STZ injection. Blood samples were collected on 0 day, 7th day, 14th day, and 21st day and till the end of the study.\textsuperscript{26} The animal groups and their dosage schedule are mentioned in Table 1.

Animals were treated for 21 days. Glucose levels were measured on day 0, that is, just before the initiation of treatment as well as on day 7th, 14th, and 21st using one touch glucometer.

**Histopathological Examination**

Under anesthesia (Pentobarbital sodium), pancreas was carefully removed from rats and immediately placed in 10%
formalin solution. Tissues were prepared using hematoxylin and eosin recoloring procedure.[13]

Statistical Analysis

The statistical analysis of all the results were carried out using one-way Analysis of Variance followed by Dunnett’s multiple comparisons using Statistical Package for the Social Sciences software (version 20) and the level of significance was determined in comparison with the control group.

RESULTS

Extraction

GS leaves and its Polyherbal composite were cold macerated with water and the extracts were dried. The percentage yield of both GS-AE and PH-AE was found to be 2.4% w/w and 4.6% w/w, respectively [Table 2]. As expected, the content of dried extract is higher in Polyherbal combination because other plants used in polyherbal combination are rich in polar phytochemicals which get extracted in higher quantities in aqueous extraction.

Chromatographic Analysis of GS-AE

Aqueous extract of GS-AE was subjected to Silica gel (60–120 μm) Column chromatography and eluted with gradient mixtures of hexane, ethyl acetate, methanol, and water of increasing polarity. Former fractions gave oily substance whereas latter fractions, that is, methanol: water (1:1) yielded crude solid on recrystallization with methanol, which showed a single spot on TLC. Rf value was found to be 0.70 in chloroform-methanol-formic acid-water (10:4:1:0.95) and visualized the spot with water.

Phytochemical Analysis of GS-AE and PH-AE

Preliminary phytochemical qualitative analysis indicated the presence of saponins and glycosides in GS-AE and saponins, glycosides, and terpenoids in PH-AE [Table 3].

Toxicity Studies

No mortality was reported even after 14 days. This indicated that the extracts were safe up to a single dose of 4000 mg/kg body weight. Hence, the selected doses for administration in experimental animals were considered 1/10th of maximum safe dose.

Antidiabetic Activity

The effects of GS-AE and PH-AE on blood glucose levels against STZ induced diabetic rats are presented in Table 4 and Figure 1. GS-AE showed significant reduction of blood glucose when compared to control group, whereas PH-AE displayed high significance when compared to standard group.

As shown in Table 5 and Figure 2, the group treated with PH-AE showed restoration of normal sugar level whereas groups treated with GS-AE and Glibenclamide still had 56.25% and 41.6% higher sugar levels after 21 days in comparison to control. Pancreatic histopathology for GS-AE and PH-AE indicated typical islet with sufficient β cell histomorphology. Relatively, in diabetic group there were atrophic and central necrotic changes seen as shown in Figure 3.

DISCUSSION

DM has been ever spreading health concern since the last century; however, now it has become more threatening because of an early onset. Numerous herbal medications have been utilized since time immemorial for the treatment of DM. GS is a famed herbal remedy for diabetes; however, GS leaves

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Table 1: Animal groups and their dosage schedule

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal saline</td>
<td>0.5 ml/kg</td>
</tr>
<tr>
<td>II</td>
<td>Normal saline+STZ</td>
<td>0.5 ml/kg+60 mg/kg</td>
</tr>
<tr>
<td>III</td>
<td>GS-AE+STZ</td>
<td>400 mg/kg+60 mg/kg</td>
</tr>
<tr>
<td>IV</td>
<td>PH-AE+STZ</td>
<td>400 mg/kg+60 mg/kg</td>
</tr>
<tr>
<td>V</td>
<td>Glibenclamide+STZ</td>
<td>5 mg/kg+60 mg/kg</td>
</tr>
</tbody>
</table>

Table 2: Yield of the extracts

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Extracts</th>
<th>Yield (percentage w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GS-AE</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>PH-AE</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Table 3: Phytochemical screening of Gymnema sylvestre aqueous extract and Polyherbal aqueous extract

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Phytoconstituents</th>
<th>GS-AE</th>
<th>PH-AE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1: Effect of GS-AE and PH-AE on blood glucose level against STZ induced Diabetes
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contain certain phytoconstituents which can damage the sense of sweet taste for a brief period, so formulating a combination which can provide a better therapeutic profile will prove to be highly beneficial and patient complaint.

In our study, chromatographic separation with methanol: water (1:1) fractions, TLC identification and phytochemical screening of GS-AE revealed the presence of Saponins with Glycosides which could be Gymnemic acid, the lead bioactive compound of GS whose Rf value of 0.70 is in accordance with the reported value.

GS-AE and its PH-AE combination (with MC, SC, and MI) were assessed for their Anti-diabetic activity (STZ-induced Diabetes). The Polyherbal extract was found to be highly effective in restoring normal sugar level. The results were highly significant ($P \leq 0.001$) when compared to standard group. Furthermore, the herbal treatment also vitalized the histological architecture of pancreas. The polyherbal extract has shown the marked presence of saponins, glycosides, and terpenoids. Based on results from antidiabetic activity, it can be suggested that these phytoconstituents not only showed their own antidiabetic effects but also ably supported the Gymnema components to yield pronounced antidiabetic effects.

**CONCLUSION**

Antidiabetic efficacy of newer Polyherbal formulation (GS, MC, SC, and MI) could be attributed to the presence of Saponins, Glycosides along with Terpenoids. Our study further showed normalization of histopathological architecture of Pancreas. This investigation suggests going for clinical research with Polyherbs to formulate a safe and potent antidiabetic drug which could help fight against diabetes which is becoming a common lifestyle disorder.

**ACKNOWLEDGMENTS**

Laboratory facilities provided by I.T.S College of Pharmacy, India, are duly acknowledged. Dr. A. P J Abdul Kalam Technical University, Lucknow, India, is acknowledged for funding the research work.

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