Research article

Anti-Diabetic Activity of Polyphyto mixture in Diabetic Rats Induced by Alloxan

Nautiyal Anuj *1, Satheesh Madhav NV 1, Bhattacharya S 2, Uniyal SN 3

1. Faculty of Pharmacy -DIT University, Dehradun, UK, India,
2. GIPER, Kashipur, Dehradun,UK
3. Surya Hospital, Dehradun, Uttarakhand

Corresponding Author : Nautiyal Anuj

ABSTRACT
The available drugs for treatment of diabetes, such as Insulin or Oral hypoglycemic agents have one or more side effects. So the search for newer anti-diabetic drugs with minimum or no side effects from herbal plants is a challenge as per WHO recommendations. Diabetes mellitus is a metabolic disorder of carbohydrate, protein and fat which are characterized by hyperglycemia, polyuria, polydipsia, and polyphagia which is deficient insulin production or ineffectiveness in insulin actions. The present study was aimed to evaluate the anti diabetic potency of Polyphyto mixture (FD39) on the blood glucose level in alloxan induced diabetic rats. Diabetic Albino Wistar strain rats were treated with standard drug Glibenclamide and test drug FD39 at 100mg. The hypoglycemic effect was determined in the rats and the efficacy of the test drug was compared to the standard drug Glibenclamide. FD39 was orally administered for 14 days in alloxan induced diabetic rats. At the end of the study duration blood glucose level and body weight were statistically analyzed. Based on these results of the study this polyphyto mixture produced a significant reduction in blood glucose levels and slight increase in the body weight when compared with diabetic control rats. And hence the present research work proved that the Polyphyto mixture possess hypoglycemic effect.

Key Words: Diabetes mellitus, Alloxan, Glibenclamide, Polyphyto mixture, Blood glucose level, Body weight, Anti-diabetic activity.

INTRODUCTION

Diabetes mellitus (DM) is a most common disorder of endocrine gland which is caused due to deficiency in insulin production or ineffectiveness of insulin produced [1]. So this a deficiency of insulin result in improper metabolism of glucose which have harmful effect in the body system, in particular the blood vessels and nerves[2]. Diabetes affects more than 171 million people worldwide and according to the resent study, this population may be increased 366 million by 2030[3].

If diabetes is not controlled by medicine it will affect the internal organs such as nephropathy, neuropathy, retinopathy etc. [4] .Although different types of oral hypoglycemic agents are available along with the insulin for diabetes treatment, there is a growing trend in herbal treatment due to the side effects occurring with allopathic medicines [5]. Plants are always been good resources for drugs and many of the currently available medications are directly or indirectly derived from plants. Most of the products obtained from plants are reported to possess anti-diabetic activity and which are widely prescribed .As they are effective, have less side effects and low cost [6,8].Hence was made to determine the anti diabetic potential of polyphyto mixture (FD39) on the blood glucose level in alloxan induced diabetic rats.

MATERIAL AND METHODS
The plant parts used in the polyphyto formulation are Ocimum sanctum (leaf) 9gm, Withania somnifera (root) 14gm, Aegle marmelos (fruit) 14gm, Trigonella Foenum graecum (seed) 27gm, Magnifera indica (bark) 30gm, Black salt 6gm were collected from in and around Dehradun district of Uttarakhand. These plants were identified and authenticated by Prof (Dr.) R.C.Dubey, Department of Botany and Microbiology, Gurukul Kangri, Vishwavidyalaya, Hardwar. The vouchers of specimen samples of the various plants were kept in the department for reference. The collected part of the plants was cleaned properly with water and was subjected to shade drying for about 8 weeks. The dried plant material was further crushed to powder and sieved (through 100 mesh) to obtain fine powder. Each of the powders was taken in different proportions as per quantity required for the formulation and thoroughly mixed together by geometrical mixing to get a homogenous mixture, stored in air tight container which was used for the study.

Animals:
Healthy wistar albino rats weight 150-200g selected by random sampling technique were used in the study. The rats were acclimatized for one week in the animal house facility. They were housed in polypropylene cages at an ambient temperature of 25±1°C with a natural dark-light cycle. They had free access to standard pellet diet and water given. The rats were fasted overnight before the starting the study but had free access for water. All experiments were conducted in day time (9:30 AM to 5:00 PM). The study was approved by Institutional Animal Ethical committee (CPCSEA registration no. -1156/AC/07/CPCSEA).

Induction of diabetes with alloxan: Diabetes was induced in rats by giving intraperitonial injection of single dose (85mg/kg) of freshly prepared alloxan monohydrate dissolved in saline solution. They were given 5% of glucose in drinking water after 1 hr to encounter any initial hypoglycemia. After 72 hrs the animals were checked for blood glucose level, those with higher than 250mg/dl were considered diabetic and used for the study [10].

Blood glucose determination - blood was obtained by snipping tail with the help of sharp razor. Blood glucose level was monitored by using Accu-Chek Active glucose monitoring kit. Each time the tail of the rats was sterilized with spirit.

Experimental Design: The selected diabetic rats were divided randomly into four groups with six animals in each group.
Group 1: Normal control received normal saline solution for 14 days
Group 2: Diabetic control received normal saline solution for 14 days
Group 3: Diabetic rats treated with standard drug glibenclamide (4 mg/ kg) orally for 14 days.
Group 4: Diabetic rats given FD39 (100 mg/kg) orally for 14 days.

The administration of the trial drug and standard drugs were carried out every day for 14days. Blood samples were collected through the tail vein just prior to and on days 14 after the drug administration and reduction in blood glucose was estimated by using glucometer and compared.

STATISTICAL ANALYSIS
The results were represented as Mean ± SD. The statistical significance was computed using One Way ANOVA followed by Tukeys multiple comparison test and compared with diabetic control group with Standard drug, FD39 where the n=6 animals in each group were used. P<0.001 was considered statistically significant.
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment (Body weight in gms)</th>
<th>After treatment (Body weight in gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (normal saline solution)</td>
<td>183.69±8.16</td>
<td>182.29±7.89</td>
</tr>
<tr>
<td>Diabetic control (normal saline solution)</td>
<td>179.73±7.00</td>
<td>171.20±8.27</td>
</tr>
<tr>
<td>Standard drug (glibenclamide 4mg/kg p.o.)</td>
<td>176.57±8.70</td>
<td>174.09±8.58</td>
</tr>
<tr>
<td>Diabetic FD39(100mg/kg p.o.)</td>
<td>182.39±7.85</td>
<td>180.24±7.31</td>
</tr>
</tbody>
</table>

Fig. No.1. Showing the diagrammatic representation of body weight of the animal groups.
<table>
<thead>
<tr>
<th>Group</th>
<th>Before treatment (Blood glucose level) mg/dl</th>
<th>After treatment Blood glucose level mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>91.5 ± 8.96</td>
<td>91.17 ± 9.15</td>
</tr>
<tr>
<td>Diabetic control (normal saline solution)</td>
<td>281.83 ± 24.72***</td>
<td>321.17 ± 20.78***</td>
</tr>
<tr>
<td>Standard drug (glibenclamide 4mg/kg p.o.)</td>
<td>302 ± 5.88***</td>
<td>100.5 ± 4.32***</td>
</tr>
<tr>
<td>Diabetic + FD39 (100mg/kg p.o.)</td>
<td>319.33 ± 24.31</td>
<td>125.83 ± 15.95***</td>
</tr>
</tbody>
</table>

Values are mean±SD, n=6 in each group. ***P<0.001 (respectively as compared to normal control) ; a3P <0.001 (as compared to diabetic control). One way ANNOVA followed by Tukeys multiple comparison test.

Figure No.2. Showing the diagrammatic representation of blood glucose level of the animal groups
RESULTS AND DISCUSSION
The commonly used chemical agent in laboratories for inducing diabetes in animal is alloxan which is an oxidized product of uric acid that causes destruction of beta cells of the pancreas by oxidation mechanism and produce Type 1 diabetes. The present study screened the anti diabetic activity of the polyphytomixture (FD39) against alloxan induced diabetic rats. The continuous treatment of the FD39 was done for a period of 14 days at 100mg/kg of body weight. Glibenclamide was the standard drug used to stimulate insulin from beta cells of islets of langerhans many years in research. So, Glibenclamide (4mg/Kg) was selected as standard drug in the study.

The results of the blood glucose level and body weights of the normal control group, diabetic control group, standard group (Glibenclamide 4mg/kg) and trial polyphytomixture (FD39) were summarized in Table No 1 and Table No 2 respectively. Data are statistically obtained by using one way ANNOVA followed by Tukeys multiple comparison test. In Table No.1, the body weight of the normal control is near about same after 14 days. However in diabetic control group was decreased from 179.73±7.00 to 171.20±8.27 after 14 days. The body weight of standard control group is near about same after 14days of treatment. The initial body weight of diabetic FD39 test group is 182.39±7.85, and after 14 days of treatment the bodyweight was about near to 180.24±7.31, there was slight increase in body weight found when compared with diabetic control group. The dose of the test Polyphyto mixture (FD39) on blood glucose level was studied in the animals. The test group showed a significant decrease in blood glucose level on alloxan induced diabetic rats when compared to diabetic control group. The initial reading of blood glucose level of FD39 was 319.33 ±24.31 before treatment. After the 14 days period FD39 produced significant reduction in the blood glucose levels (125.83 ±15.95). In standard drug group initial blood glucose level was 302 ±5.88 and the after 14 days it was 100.5 ±4.32 which showed that the standard drug had produced maximum anti-diabetic effect. The diabetic control group showed rise in blood glucose level throughout the study period. Initially the blood glucose level of diabetic control group was 281.83±24.72 and after 14 days of study period the blood glucose level was increased to 321.17 ±20.78. The results of blood glucose level in rats were summarized in Table No.2. And on the basis of the results, it was observed that there was an significant reduction in blood glucose level by Polyphyto mixture (FD39) in alloxan induced diabetic rats. The anti-diabetic activity of this FD39 could be due to the increased release of insulin from beta cells of the pancreas or may be due to potentiating effect of insulin. Treatment of Polyphyto mixture (FD39) in diabetic rat also showed the significant weight gain property which proved its efficacy of this Polyphyto mixture in treating diabetic patients successfully.

CONCLUSION
Polyphyto mixture (FD39) is a mixture of six herbal plants and it is found to be more effective in the treatment of diabetes mellitus as determined by its statistically significant p-value < 0.001 in alloxan induced diabetic rats. The mechanism of anti-diabetic activity of this Polyphyto mixture may be due to enhancing the effect of insulin and by stimulating the insulin secretion from beta cells of pancreas. Hence this study suggests that this Polyphyto mixture has a potent anti diabetic effect which could be used for the management of diabetes effectively.

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References


