EVALUATION OF ANTIDIABETIC ACTIVITY OF ALCOHOLIC EXTRACT OF ALOE BARBADENSIS, MOMARDICA CHARANTIA, TRIGONELLA FOENUM-GRAECUM AND THEIR COMBINATIONS USED IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The objective of the study is to demonstrate the antidiabetic activity of the alcoholic extracts of Momordica Charantia, Aloe Barbadensis and Trigonella Foenum-Graecum and their combination were used in alloxan-induced diabetic albino rats. The powdered leaves of Momordica Charantia, Aloe Barbadensis and Trigonella Foenum-Graecum were successively extracted in 100-150ml each of alcohol by using a soxhlet apparatus. The rats after alloxination were given 5%w/v glucose solution in feeding bottles for the next 24hrs in their cages to prevent hypoglycaemia. After 72hrs, rats with fasting blood glucose levels greater than 200mg/dl were selected and used for the study. The blood samples were collected through tip of tail vein at 0, 1, 2, 4 and 8 hrs respectively after administration. The treatment was continued for next 22days. The blood glucose level was estimated at various intervals by using glucometer. The difference observed between the initial and final fasting blood glucose levels of extract treated hyperglycaemic rats revealed the antihyperglycemic effect of Momordica Charantia, Aloe Barbadensis and Trigonella Foenum-Graecum and their combination throughout the period of study. The effect of three different extracts and their combinations are compared to that of reference standards, glibenclamide was found to be significant. In conclusion, these extracts showed significant anti-diabetic effect in diabetic rats after I.P administration. The drug has the potential to act as an anti-diabetic drug in combination therapy.
KEYWORDS: Alloxan, Anti-diabetic activity, Glibenclamide, Momordica Charantia, Aloe Barbadensis and Trigonella Foenum-Graecum.

INTRODUCTION
Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders.\(^1\text{-}^4\) It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be. Diabetes is still a serious health problem all over the world since it is associated with increased morbidity and mortality rate. When compared with the general population, mortality and morbidity increase in diabetes is mainly due to the associated chronic complications both specific (microvascular) and nonspecific (macrovascular). Since the disease prevails in both genders and in all age groups, the general public has a concern about its control and treatment.\(^5\)

In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of few Indian medicinal plants.

The Pharmacognostical evaluation of Indian medicinal plants viz., \textit{Aloe Barbadensis}, \textit{Momordica Charanti}, \textit{Trigonella Foenum-Graecum} and combination were studied which include the morphological and physicochemical studies. Considering the aforesaid, the study is aimed to demonstrate the antihyperglycemic activity of the alcoholic extracts of \textit{Momordica charantia}, \textit{Aloe barbidensis}, \textit{Trigonella foenum} and their combinations were used alloxan induced diabetic model in rats.
MATERIALS AND METHODS

Drugs and Chemicals
Drugs and Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India.

Experimental animals
Healthy adult albino wistar rats weighing 200-250 grams of either sex were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of experiment, after 72 hours of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house and the room temperature was maintained at 27°C. Animal studies had approval of IAEC.

Plant Material Collection
The leaves of Aloe Barbadensis, Momordica Charantia and Trigonella Foenum-Graecum were collected from Geethanjali College in the month of December. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.[6]

Preparation of plant extracts
The powdered Leaves of Aloe barbidensis, Momordica charantia and Trigonella Foenum-Graecum were successively extracted in 100-150 ml each of alcohol by using Soxhlet extractor. The plant material was suspended in the main chamber of Soxhlet extractor which was then placed onto a flask containing the extraction solvent.[7-9] The Soxhlet was then equipped with a condenser. The flask was heated; the solvent evaporated and moved up into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the sample. This extraction process kept for 8 hrs at 20-40°C. At the end of the hot extraction process each extract was filtered. The filtered extract was dried in oven to remove remaining moisture, if present, and finally weighed and sealed up for further use.

The alcoholic extracts of Aloe Barbadensis, Momordica Charantia and Trigonella Foenum-Graecum suspended in water in presence of 3% v/v Tween-80 solution.
All the drugs were administered i.p for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

Assessment of anti-diabetic activity in alloxan induced diabetic rats

Induction of Diabetes

Albino wistar rats of either sex weighing 200-250 g were selected for the study. All the animals were allowed free access to water and pellet diet and maintained at room temperature in rat cages.

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 12 hour fasted rats\textsuperscript{[10-13]} by single intraperitoneal injection of 120 mg/kg body weight of freshly prepared alloxan in normal saline.

The rats after alloxanization were given 5% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. After 72 hours rats with fasting blood glucose levels greater than 200 mg/dl were selected and used for further studies.

All the animals were observed for seven days for consistent hyperglycemia (fasting blood glucose level greater than 200 mg/dl and lesser than 400 mg/dl) and such animals were selected and divided into six groups of four each and used for the study of the following experimental models.\textsuperscript{[14-17]}

Table-1. Group Classification.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal control received distilled water</td>
<td>10ml/kg</td>
</tr>
<tr>
<td>Group 2</td>
<td>Standard group received Glibenclamide</td>
<td>10ml/kg</td>
</tr>
<tr>
<td>Group 3</td>
<td>Alcoholic extract of \textit{Aloe Barbadensis}</td>
<td>200</td>
</tr>
<tr>
<td>Group 4</td>
<td>Alcoholic extract of \textit{Momordica Charantia}</td>
<td>200</td>
</tr>
<tr>
<td>Group 5</td>
<td>Alcoholic extract of \textit{Trigonella Foenum-Graecum}</td>
<td>200</td>
</tr>
<tr>
<td>Group 6</td>
<td>Alcoholic extract of \textit{Aloe Barbadensis, Momordica Charantia and Trigonella Foenum-Graecum}</td>
<td>200</td>
</tr>
</tbody>
</table>

Effect of alcoholic extracts of \textit{Aloe barbidensis}, \textit{Momordica charantia} and \textit{Trigonella Foenum-Graecum} and their combination on blood glucose levels in alloxan induced diabetic rats

All the animals of above groups were administered as per treatment protocol mentioned above. The blood samples were collected by retro orbital puncture at 0,1,2,4 and 8 hour after
the administration. The treatment was continued for next 22 days. Again blood samples were also collected on 4th, 7th, 14th and 21st day after 1 hour administration for sub acute study. All blood samples were collected from tail artery of the rats at different time intervals. Determination of blood levels was done by glucose oxidase principle using the One Touch Basic (Lifescan, Mulpital CA instrument) and the result were expressed as mg/dl.

**Oral glucose tolerance test (OGTT) in alloxan induced diabetic rats.**

On the 8th, 15th and 22nd day OGTT was carried out on the same alloxan induced diabetic animals used for assessment of anti-diabetic activity studies.

**Procedure**

All the animals in each group were administered 2g/kg of glucose one hour after extract/Glibenclamide/ vehicle administration. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hour after the administration of the glucose load. Serum was treated with solutions of GOD/POD kit and according to procedure blood glucose levels were measured under by Biochemical analyzer.

**RESULTS**

**Acute toxicity testing**

Acute toxicity studies revealed that the alcoholic extracts of *Momordica Charantia, Aloe Barbidensis, Trigonella Foenum* and their combinations were safe up to 2000 mg/kg of body weight and approximate LD 50 is more than 2000 mg/kg. No lethality or any toxic reactions was observed up to the end of the study period.

**Anti-diabetic activity in alloxan induced diabetic rats**

Fasting blood glucose levels (FBGL) in normal rats were in range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, I.P.) had increased the FBGL to range of 252-266 mg/dl after 72 hours. These values on subsequent days got stabilized by day seven on an average between 255 mg/dl.

Changes in the fasting blood glucose levels in different groups are tabulated in Table No: 1. this data shown that blood glucose level of normal control animals has maintained throughout the study period. The group 1 which is the diabetic control group has shown significant increase in fasting blood glucose levels during this 21st day study period. The
group 2 Glibenclamide (10mg/kg) treated group has shown (p<0.005) significant decrease in fasting blood glucose level during 7th, 14th and 21st day of study period.

**Effect of ALEM C, ALEAB, ALETF and ALECO on antidiabetic activity in alloxan induced diabetic rats**

The animals treated with 200mg/kg of ALEM C, ALEAB, ALETF and ALECO shown significant decrease (P<0.005) in FBGL on 7th, 14th and 21st day of treatment when compare to other groups of animals. The alcoholic extract has reduced more (%) in FBGL when compared to standard group. The detailed results are summarized in TableNo: 2.

**Table No: 2 Effect of ALEM C, ALEAB, ALETF and ALECO on fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose level(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Normal control</td>
<td>-</td>
<td>100±1</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>10</td>
<td>253±2</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10</td>
<td>98±1</td>
</tr>
<tr>
<td>ALEM C</td>
<td>200</td>
<td>85±2</td>
</tr>
<tr>
<td>ALEAB</td>
<td>200</td>
<td>91±2</td>
</tr>
<tr>
<td>ALETF</td>
<td>200</td>
<td>90±2</td>
</tr>
<tr>
<td>ALECO</td>
<td>200</td>
<td>92±1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n=3. Significant values were compared with P<0.005. Normal control Vs all groups. Paranthesis indicates % reduction in BGL.

**Oral glucose tolerance test (OGTT) on 1st, 8th, 15th & 22nd day**

ALEMC, ALEAB, ALETF and ALECO (200 mg/kg) significantly (P<0.005) suppress the rise in FBGL after glucose load (2g/kg) in rats, at first half-an-hour and upto 2hr time period as compare with other groups extract glibenclamide on 15th day. While ALEM C, ALEAB, ALETF and ALECO produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.005) significant suppression in FBGL rise at 1st, 8th, 15th & 22nd day normalized FBGL within 2hr. The detailed results are summarized in TableNo: 3.
Table No: 3 Effect of extracts of *E. aureum. Linn* on 22\textsuperscript{nd} day in normal rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose level(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} day</td>
</tr>
<tr>
<td>Normal control</td>
<td>-</td>
<td>101±2</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>10</td>
<td>259±3</td>
</tr>
<tr>
<td>Std (Glibenclamide)</td>
<td>10</td>
<td>107±2</td>
</tr>
<tr>
<td>ALEM C</td>
<td>200</td>
<td>466±1</td>
</tr>
<tr>
<td>ALEAB</td>
<td>200</td>
<td>199±2</td>
</tr>
<tr>
<td>ALET F</td>
<td>200</td>
<td>160±3</td>
</tr>
<tr>
<td>ALECO</td>
<td>200</td>
<td>149±2</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. n=3. Significant values were compared with P<0.005. Normal control Vs all groups. Parenthesis indicates % reduction in BGL.

DISCUSSION

The present study was aimed at discovering the antidiabetic activity of alcoholic extracts of *Momordica Charantia, Aloe Barbidensis, Trigonella Foenum* and their combinations at a dose of 200mg/kg showed significant effect on glucose tolerance and the extracts also showed reduction in fasting blood glucose levels in normal and alloxan induced diabetic rats. These findings indicate that the extracts might be producing hypoglycaemic effect by a mechanism independent from the insulin secretion e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus in animals. It induces diabetes by dose dependent destruction of β-cells of islets of langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 22 days treatment. The difference observed between the initial and final fasting blood glucose levels of extract treated hyperglycemic rat’s revealed antihyperglycemic effect of *Momordica Charantia, Aloe Barbidensis, Trigonella Foenum* and their combinations throughout the period of study. The effect of three different extracts and their combinations are compared to that of reference standard, glibenclamide was found to be significant.
CONCLUSION

The Pharmacognostical evaluation of Indian medicinal plants viz., *Aloe Barbadensis*, *Momordica Charanti*, *Trigonella Foenum-Graecum* and combination were studied which include the morphological and physicochemical studies in order to establish the quality, safety and efficacy.

The data of the blood glucose level of rats treated with Alloxan (150mg/kg body weight) produced diabetes within 72 hours. After 72 hours of Alloxan administered the blood glucose levels of rats were observed. It was observed that significant lowering of sugar in alcoholic extract. The administration of ALEAB, ALEMC and ALETF at a dose of 200 mg/kg showed significant anti-hyperglycaemic effect at 21st day which was evident from the 1st day onwards as compared to standard. The alcoholic extract of three extracts combination has showed better efficacy than the individual extract in all the treated extract.

Results of anti-diabetic activity of extracts established the scientific basis for the utility of these plants in the treatment of diabetes. The alcoholic extracts have shown significant reduction in blood glucose levels in alloxan induced diabetic rats and produced maximum anti-diabetic activity and are higher than the hypoglycaemic activity of Glibenclamide in the diabetic rats. Therefore it is obvious that the fractionation with alcohol has enriched the active principles. In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Alcoholic extracts in combination has reduced the glucose levels, in prolonged treatment study. In conclusion, these extract showed significant anti-diabetic effect in diabetic rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of these plants in the treatment of diabetes stands confirms.

REFERENCES


