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EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF THE ALCOHOLIC EXTRACTS OF *MOMORDICA CHARANTIA, TRIGONELLA FOENUM AND* THEIR COMBINATIONS IN ANIMALS

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ABSTRACT

Diabetes is one of the most common non-communicable diseases and a serious lifelong condition appearing worldwide. The study is aimed to demonstrate the antihyperglycemic activity of the alcoholic extracts of *Momordica charantia, Trigonella foenum, and* their combinations were used alloxan induced diabetic model in rats. The powdered Leaves of *Trigonella foenum and Momardica Charantia* were successively extracted in 100-150ml each of alcohol by using a Soxhlet extractor.The rats after alloxanization were given 5% w/v glucose solution in feeding bottles for the 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 the study. The blood samples were collected by retro orbital puncture at 0,1,2,4 and 8 hours after the administration. The treatment was continued for next 22 days.The blood glucose level was estimated at various time intervals by subjecting the collected blood to cold centrifugation for serum separation. Serum obtained was used for estimating the glucose level using GOD/POD (span) kit. The difference observed between the initial and final fasting blood glucose levels of extract treated hyperglycemic rats revealed the antihyperglycemic effect of *Momordica Charantia, 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 standards, glibenclamide was found to be significant. In conclusion, these extracts showed significant anti-diabetic effect in diabetic rats after oral administration.

Keywords: ***Momordica charantia, Trigonella foenum, Momordica charantia, Trigonella foenum*** **Diabetes**

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INTRODUCTION

Diabetes is one of the most common non-communicable diseases and a serious lifelong 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[1-4]. 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. Plants have been an exemplary source of medicine and many of the currently available drugs have been derived directly or indirectly from them. India has about 45,000 plant species and the research conducted in the last few decades on traditional plants reports the ethno-botanical information of about 800 plants that may possess anti-diabetic potential. Very few of the traditional plant treatments for diabetes have received scientific scrutiny to assess their efficacy despite the fact that World Health Organisation has recommended that pharmacological and a scientific examination of such plants warrants attention (WHO, 1980). Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently. The hypoglycemic effect of some herbal extracts has been confirmed in animal and human models of Type 2 diabetes. There are reports of using herbal extracts for the treatment of diabetes mellitus in humans. Adverse effects are indeed a cause of concern, however, available evidence suggests that herbal medicines are relatively safe[5-7] The potential role of the medicinal plants as hypoglycemic agents has been reviewed by several authors. Many Indian medicinal plants are reported to be useful in diabetes. Several of the most studied and commonly used medicinal herbs for diabetes include *Ginseng species, Momordica charantia* (Bitter Melon), *Trigonella foenum graecum* (Fenugreek), *Gymnema sylvestre* (Gurmar), *Allium cepa* (Onion) and *Allium sativum* (Garlic), *Petrocarpus marsupium, Vaccinium myrtillus* (Bilberry), *Atriplex halimus* (Salt Bush), *Aloe vera*. A wide array of plant derived active principles representing numerous chemical compounds like alkaloids, glycosides, polysaccharides, guanidine, steroids, carbohydrates, glycopeptides, terpenoids etc have demonstrated activity consistent with their possible use in the treatment of NIDDM. Medicinal plants have been used for diabetes safely and with reasonable success. Despite the great strides that have been made in understanding and management of diabetes mellitus, serious complications continue to confront patients and physicians. Therefore, search for anti-diabetic plants which are already used but in the combination of that are used in this study to show an additive effect when compared to that of individual drug therapy. Momordica charantia L. (Cucurbitaceae), revealed the presence of potassium, sodium, calcium as well as zinc. Other elements found present in the leaf include magnesium, iron, manganese and copper. Vitamin A (β-carotene), vitamin E (α-tocopherol), folic acid, cyanocobalamin and ascorbic acid were present. Trace amounts of some other vitamins such as niacin (B3), pyridoxine (B6) cholecalciferol (Vitamin D) and phylloquinone (Vitamin K). Phytochemicals like alkaloids, tannins, flavonoids, saponins and glycosides were also found present. Trigonella foenum-graecum L (Fabaceae) contains Trigogenin, neotrigogenin, diosgenin, yamogenin, gitogenin, 4-hydroxyisoleucine, vitexin, isovitexin, saponaretin, homoorientin, vicenin-1, vicenin-2 and two flavonoid glycosides quercetin and luteolin and steroidal saponins have been isolated from seeds. Seeds also contain essential oil, fixed oil, fatty acids, proteins, large number of amino acids, carbohydrates, vitamins A, B1 and C, nicotinic

acid, minerals and several coumarins. Saponin isolated from stems on hydrolysis yield a sapogenin, while that isolated from leaves gave diosgenin, tigogenin and gitogenin. Considering the aforesaid, the study is aimed to demonstrate the antihyperglycemic activity of the alcoholic extracts of *Momordica charantia, Trigonella foenum and* their combinations were used alloxan induced diabetic model in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Alloxan monohydrate (Quali Kems Fine Chem Pvt, Ltd, Vadodara), Alcohol (ChangshuYangyuan Chemicals, China), Glibenclamide (Sanofi India Ltd, Ankleshwar).

Experimental animals

Healthy adult albino wistar rats weighing 200-250grams 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 the experiment, after 72hours of fasting for 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 the approval of IAEC.

Plant Material Collection

The leaves of *Momardica Charantia and Trigonella Foenum-Graecum* were collected local area 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[8].

Preparation of plant extracts

The powdered Leaves of *Momordica charantia* and *Trigonella Foenum-Graecum* were successively extracted in 100-150ml each of alcohol by using a Soxhlet extractor. The plant material was suspended in the main chamber of Soxhlet extractor which was then placed into a flask containing the extraction solvent[9-11]. 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 8hrs at 20-40^{0}C. At the end of the heat extraction process each extract was filtered. The filtered extract was dried in an oven to remove remaining moisture, if present, and finally weighed and sealed up for further use.

Preparation of extracts

The alcoholic extracts of *Momardica 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 timely preparation of the extracts was prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.

Induction of Diabetes

Alloxan was dissolved in normal saline immediately before use. Diabetes was induced in 16 hour fasted rats 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 the 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. Group 1 received Normal control received distilled water, Group 2 received, Standard group received Glibenclamide, Group 3 received Diabetic control, Group 4 received an Alcoholic extract of *Trigonella Foenum-Graecum,* Group 5 received an Alcoholic extract of *Momardica Charantia,* Group 6 received an Alcoholic extract of *Momardica Charantia and Trigonella Foenum-Graecum.* 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 six each and used for the study of the following experimental models[12-15]. All the animals of the 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. Blood glucose level was estimated at various time intervals by subjecting the collected blood to cold centrifugation for serum separation. Serum obtained was used for estimating glucose level using GOD/POD (span) kit. 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. All the animals in each group were administered 2g/kg of glucose one hour after extract/ Glibenclamide/ vehicle administration[16-19]. The blood samples were collected by retro orbital puncture at 0 hour, 0.5 hour, 1 hour, 1.5 hour and 2 hours 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.

Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test (0.01).

RESULTS & DISCUSSION

The present study was aimed at discovering the antidiabetic activity of alcoholic extracts of *Momordica Charantia, 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 the hypoglycaemic effect by a mechanism independent from the insulin secretions 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 a 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.

Fasting Blood Glucose Level (FBGL) in Alloxan induced diabetic rats

The animals treated with 200mg/kg of ALEMC, ALEAB, ALETF and ALECO shown a significant decrease (P<0.1) in FBGL on 7th, 14th and 21st day of treatment when compare to other groups of animals. The aqueous extract has reduced more (%) in FBGL when compared to alcoholic extracts except standard group.

Table 1
Effect of ALEMC, ALETF and ALECO on fasting blood glucose level (FBGL) in Alloxan induced diabetic rats.

Treatment	Dose(mg/kg)	Blood glucose level(mg/dl)			
		0 day	7th day	14th day	21st day
Normal control	-	100±1	100±1	96±1	97±2
Diabetic control	10	253±2	256±3	264±2	271±1
Glibenclamide	10	98±1	96±3	80±1	72±2
ALEMC	200	85±2	84±1	72±2	60±1
ALETF	200	90±2	88±3	85±1	83±2
ALECO	200	92±1	84±2	72±3	60±2

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.1. Normal control Vs all groups.

Oral glucose tolerance test (OGTT) on 16th day-

ALEMC, 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 ALEMC, ALETF and ALECO produced significant reduction in FBGL. Glibenclamide (10mg/kg) showed (P<0.1) significant suppression in FBGL rise at 1st, 8th, 15th& 22nd day normalized FBGL within 2hr. The detailed results are summarized in TableNo: 2

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Table 2

Effect of extracts of ALEMC, ALETF and ALECO on 22nd day in normal rats.

Traetment	Dose (mg/kg)	Blood glucose level(mg/dl)			
		1st day	8th day	15th day	22nd day
Normal control	**-**	101±2	142±4	132±2	118±2
Diabetic control	**10**	259±3	383±5	339±2	290±2
Std(Glibenclamide)	**10**	107±2	125±2	115±2	112±1
ALEMC	**200**	466±1	457±4	394±2	340±1
ALETF	**200**	160±3	148±2	125±4	113±2
ALECO	**200**	149±2	135±3	96±1	85±3

Values are expressed as mean ± S.E.M. n=6. Significant values were compared with P<0.1. Normal control Vs all groups. The difference observed between the initial and final fasting blood glucose levels of extract treated hyperglycemic rats revealed antihyperglycemic effect of *Momordica Charantia, 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 standards, glibenclamide was found to be significant.

SUMMARY AND CONCLUSIONS

In current scenario, herbs are the potent sources of medicines used in the treatment of various diseases 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 m edicinal plants viz., *Momardica Charanti, Trigonella Foenum-Graecum and combination were* studied which include the morphological and physicochemical studies. The morphological studies of species, plant part were studied which will be beneficial for the validation and assessment of quality control parameters of these plants to find out the presence of adulterant if any in order to establish the quality, safety and efficacy. The data of the blood glucose level in 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 ALEMC and ALETF at a dose of 200 mg/kg showed significant anti-hyperglycaemic effect on 21st day, which was evident from the 1st day on wards as compared to standard The alcoholic extract of three extracts combination has showed better efficacy than the individual extract in all the treated extract. The anti-hyperglycaemic effect of the extract on the fasting blood sugar levels on diabetic rats is shown in table. The decreasing blood glucose levels are comparable with that of 10 mg/kg of Glibenclamide. The Glibenclamide (10 mg/kg body weight) shows significant effect on compare to the initial and more significant effect on the 7th Day compare to the initial. The

alcoholic extracts (200mg/kg body weight) shows significant (P*<0.1), effect. 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 have reduced the glucose levels, in prolonged treatment study. In conclusion, these extracts 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.

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