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Original Research Article

Study of the oral hypoglycemic activity of *Moringaoleifera* leaves alone and in combination with Glibenclamide in streptozotocin induced diabetic albino rats

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ABSTRACT

Background: Oringaoleifera is a widely used plant with high medicinal value, well known for its pharmacological actions and is used in various conditions. It has been reported to have many biological properties like anti-inflammatory, antimicrobial, antispasmodic, antitumour including antidiabetic activity.
Methods: The study was carried out in Wistar albino rats with body weight 150-250gms. Diabetes was induced by injecting Streptozotocin intraperitoneally-dose 55 mg/kg BW. Animals were divided into 5 groups with 6 animals in each group. First group (Control) was given 2% gum acacia. Other 4 groups were induced diabetes by giving Streptozotocin. Diabetic control group received gum acacia (0.5 ml), Standard group received Glibenclamide (0.5mg/kg BW), Test group received *Moringaoleifera* extract (300mg/kg) and Test+ Standard group receiving combination of *Moringaoleifera* and glibenclamide at half the above doses. All drugs were given orally for 28 days and blood glucose levels analyzed using Glucometer on Day 0 before drug and on D1, D3, D7, D14, D21, and D28. Data were statistically analyzed by ANOVA and Tukey's Post Hoc test.
Results: Hypoglycemia produced by *Moringaoleifera* extract was significant ($p<0.001$) when compared to diabetic control group from day 7 to day 28. The percent reduction of blood glucose level was 52.9% as compared to Glibenclamide group 61.3%. The combination group also showed significant hypoglycemic activity the percentage reduction being 56.44%.
Conclusions: Thus, *Moringaoleifera* decreased blood glucose level efficaciously as compared to diabetic control group and similar to standard group at $p<0.001$.

Keywords: Diabetes mellitus, Hypoglycemia, *Moringaoleifera*, Streptozotocin

INTRODUCTION

Diabetes mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by complex interaction of genetic and environmental factors. Depending on the etiology of DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, increased glucose production etc. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems andis the leading cause of end stage renal disease, non traumatic lower extremity amputation, adult blindness and also predisposes to cardiovascular diseases. With an increasing incidence worldwide, DM will be a leading cause of morbidity and mortality for the foreseeable future.[1]

Once considered primarily as a risk factor for heart disease, diabetes has now become a high profile public health concern in its own right, due to the escalating

epidemic of diabetes in older people, and emergence of type 2 diabetes in children.

The prevalence of diabetes mellitus is growing rapidly worldwide and is reaching epidemic proportions.[2] It is estimated that there are currently 285 million people worldwide and this number is set to increase to 438 million by the year 2030.[3]

Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle-aged people.

It is now among the five leading causes of death due to disease in most of the countries and its huge personal and socio economic costs are increasingly being recognized as a major global health and societal problem, particularly by the World Health Organization and World Bank.[4]

Despite the availability of many anti diabetic medicines in the market, diabetes and its related complications like atherosclerosis, neuropathy, nephropathy and retinopathy are continued to be major medical problems. So,continuous attempt is on for better oral hypoglycemic agents in the treatment of diabetes. Medicinal plants constitute an important source of potential therapeutic agents for diabetes and hyperlipidemia. Extracts of various plant materials capable of decreasing blood sugar have been tested in experimental animal models.

Moringaoleifera (MO), commonly called 'drumstick' belongs to family *Moringaceae*, a multipurpose tree found almost all over Asian and African countries and its fruit and leaves are consumed as food by the people.[5] It contains several phytochemicals, some of which are of high interest because of their medicinal values.[6] Most parts of the plant possess antimicrobial activity. They are well known for their pharmacological actions and are used in traditional treatment of diabetes mellitus, hepatotoxicity, rheumatism, venomous bites and for cardiac stimulation.[7]

*M. oleifera*have been claimed to possess hypoglycemic effect in Indian traditional system of medicine. The leaves of other species of *Moringa (M.stenopetala)* are used in Ethiopia for treating diabetes and has been explored for their hypoglycemic action.[7]

Moringaoleifera as a rich source of ascorbic acid helps in insulin secretion. It is interesting to note that certain nutrients like vitamins B_1, B_2, B_{12}, pantothenic acid, vitamin C, protein and potassium- along with small frequent meals containing some carbohydrate- can actually stimulate production of insulin within the body.[8]

Moringaoleifera has been shown to naturally boost the immune system, which usually becomes compromised in those who suffer from type 1 and type 2 diabetes. *Moringaoleifera* has also been shown to possess many key anti-inflammatory benefits; diabetes often causes endothelial damage which can be managed through anti-inflammatory supplements.[8]

A study has shown that Benzylamine is found in *Moringaoleifera*. In mammals, Benzylamine is metabolized by semicarbazide-sensitive amine oxidase (SSAO) to benzaldehyde and hydrogen peroxide. This product (H_2O_2) has insulin-mimicking action (in vitro insulin like action found on adipocytes has been proven), and is involved in the effects of benzylamine on human adipocytes, stimulation of glucose transport and inhibition of lipolysis.[9]

Moringaoleifera holds so much promise for those who suffer from diabetes. There are no side effects associated with *Moringaoleifera* use, meaning that it is a safe, natural way for people to manage their blood sugar and care for their diabetes symptoms.

It was therefore considered worthwhile to investigate the ethanolic extract of *Moringaoleifera* leaves for its hypoglycemic potential in Streptozotocin induced diabetic albino rats. This may prove to be beneficial for the development of novel therapeutic approaches to diabetes, alone and in combination with available antidiabetic drugs.

Objectives

- To scientifically investigate the effect of ethanolic extract of *Moringaoleifera*leaves on glycemic level in streptozotocin induced diabetic albino rats.
- To compare the hypoglycemic effect of ethanolic extract of *Moringaoleifera* leaves with that of the standard drug Glibenclamide used in the treatment of diabetes mellitus.
- To evaluate the combined effect of ethanolic extract of *Moringaoleifera* leaves and glibenclamide.
- To compare the hypoglycemic activity of combination of ethanolic extract of *Moringaoleifera* leaves and glibenclamide with *Moringaoleifera* alone.

METHODS

Preparation of extract

Leaves of *Moringaoleifera* was obtained in sufficient quantity from JSS ayurvedic hospital, Mysore. They were dried in shaded area and powdered and then extract taken using Soxhlet apparatus.

The extract was used at dose of 300 mg/kg10[7]. The solution was freshly prepared by dissolving the ethanolic extract in gum acacia on the respective days of experiment and administered by oral route.[10]

Animals

Animals used were adult healthy albino rats, of Wistar strain, weighing between 150-250gm of either sex. The

rats were inbred in the central animal house of the Department of Pharmacology, J.S.S Medical College, Mysore, under suitable conditions of housing, temperature, ventilation and nutrition. The animals were fed with commercial laboratory food and water ad libitum. They were maintained at a temperature of 24-27^{0}C with relative humidity of 30-70% with 12 hr light dark cycle.

Induction of diabetes

Following an overnight fast, 30 rats were injected intraperitonially, with freshly prepared Streptozotocin (dissolved in sodium citrate buffer) under aseptic precautions in a dose of 55mg/kg body weight 3 days before the experiment.[11,12] Animals were carefully observed for first 24 hours following the injection for any evidence of allergic reactions, behavioural changes, convulsions and hypoglycemic attacks.

Blood glucose level was recorded everyday morning at around 9.00 am for 3 days. Animals developed stable hyperglycemia after 3 days. Only those animals with blood glucose level more than 250mg/dl were selected for the study.

Grouping of animals

- *Group 1:* Normal control: 0.5ml 2% Gum acacia (oral)
- *Group 2:* Diabetic control: Streptozotocin i.p. + 2% Gum acacia (oral)
- *Group 3:* Standard: Streptozotocin i.p. + 0.5mg/kg BW Glibenclamide13 (oral)
- *Group 4:* Test: Streptozotocin i.p.+300mg/kg BW Moringaoleifera (oral).
- *Group 5:* Test + Standard: Streptozotocin i.p. + 0.25mg/kg BW + Glibenclamide (oral) + 150mg/kg Moringaoleifera (oral) (suboptimal doses of both drugs as they are used in combination).

Estimation of body weight

Body weight of the individual rats was measured on the respective days before blood glucose estimation on 0, 1, 3, 7, 14, 21 and 28th day.

Method of blood collection

Blood was collected by rat tail snipping method. The animal was placed in a suitable rest rainer, the tip of the tail was cleaned with a disinfectant and was cut by a scissor under aseptic precautions. Blood drop which was formed was used for blood glucose estimation.

Estimation of blood glucose

Blood was collected from 18 hr fasted rats at 1hr after each dose administration of the respective drugs by rat tail snipping method and fasting blood glucose was estimated by OPTIMUM EXCEED glucometer on 0, 1, 3, 7, 14, 21 and 28th day.

Statistical analysis

The data obtained from the present study was subjected to statistical analysis. Mean and standard deviations were calculated for each group. One way ANOVA was used for multiple group comparisons followed by post hoc Tukey's test for statistical significance between groups. P values less than 0.05 was considered to be significant.

RESULTS

Control group

Mean values of blood glucose levels range between 81.00 on Day 0 to 79.5 mg/dl on Day 28 without much of variation during the study (Table 1 and Figure 1).

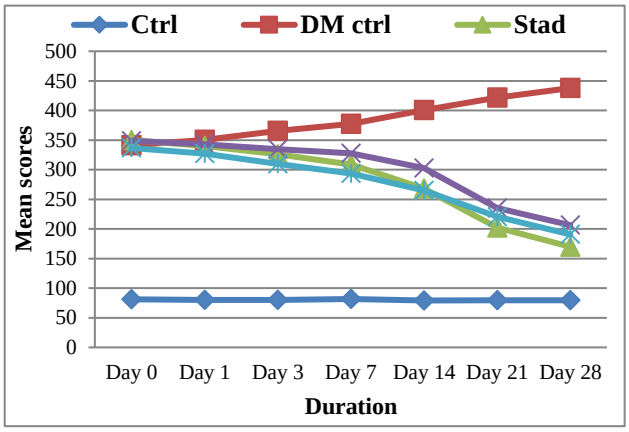

Figure 1: Variation in blood glucose levels from day 0 to day 28 in study groups.

Diabetic control group

In untreated diabetic control rats, the blood glucose levels increased gradually from 341.2mg/dl on Day 0 to 350.3mg/dl on Day 1 and later to 437.8mg/dl on Day 28 showing an increment in the blood glucose value on all the reading days. (Table 1, Figure 1). The percentage increase in BGL from Day1 to Day 28 was 22.1%.

Standard group

In the Glibenclamide treated rats the mean blood glucose level was 349.8mg/dl on Day0, which reduced to 339.8mg/dl on Day 1 (4.1%), and then later it steadily decreased to 326.2 on Day 3 (10.71%), 308.5 on Day 7 (18.22%), 268.2 on Day 14 (33.1%), 202.2 on Day 21 (52.1%) and showed maximum reduction of 66.3% on Day 28 where blood glucose level reduced to 169.8mg/dl.

The body weight of this group of rats decreased following STZ injection but with continuous treatment with glibenclamide, their body weight gradually increased and

reached almost the initial weight by the end of day 28 (Table 2).

The difference in the blood glucose levels between Day 0 to Day 1 is 10mg/dl, between day 0 and day 3 is 23.6mg/dl showing that the standard drug Glibenclamide has immediate hypoglycemic action hence there is immediate lowering of the blood glucose and the overall difference between CBG levels between Day 0 to Day 28 is 180mg/dl showing consistent reduction, thus this shows the total oral hypoglycemic efficacy of Glibenclamide in the study.

Table 1: Mean+SD values of Blood glucose levels in different groups.

Groups	D0	D1	D3	D7	D14	D21	D28
Control	81±5.762	80.17±4.75	80±5.621	81.67±6.121	79.17±5.529	79.67±3.141	79.5±3.782
Diabetic control	341.2±24.64	350.3±24.45	365.3±21.74	377.2±22.83	400.8±35.49	421.7±32.64	437.8±34.03
Standard	349.8±21.12	339.8±22.42	326.2±22.45	308.5±26.32	268.2±26.39	202.2±13.89	169.8±13.3
Test	348.8±22.56	342.8±23.34	334.5±22.77	327.7±21.78	303±18.19	235.3±28.72	206.3±12.74
Test+ Standard	336.7±19.45	327±20.67	310±21.91	293.7±17.82	264.8±20.53	220.7±34.42	190.7±20.65
Gr.1 (Control) V/S GR. 2, 3, 4, 5	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001	P <0.001

D 0 = before giving the drug
D 1, D 3, D 7, D 14, D 21, D 28 = 1st, 3rd, 7th, 14th, 21st, 28th days of administration of the drugs respectively

Table 2: Mean values of body weight of rats in different groups on different days.

Groups	Before STZ	D0	D1	D3	D7	D14	D21	D28
Control	190	190	190	200	200	210	200	190
Diabetic control	210	180	170	150	160	160	170	170
Standard	200	170	170	180	190	190	200	210
Test	200	180	180	160	170	170	190	200
Test+ Std	190	170	160	160	180	190	190	210

Test group

In the *Moringaoleifera* treated group the blood glucose level on Day 0 was 348.8mg/dl, which reduced to 342.8mg/dl on day 1 (2.2%), 334.5 on day 3 (8.5%), 327.7 on day 7 (13.3%) showing slow reduction in blood glucose values (indicating delayed onset of action), and later there was persistent reduction of blood glucose level from 303 on day 14 (24.25%), 235.3 on Day 21 (44.29%) to 206.3 on Day 28 (52.9%) which was statistically significant compared to diabetic control (Table 1 and Table 3).

Their body weight decreased following STZ injection till day 3 after which they showed improvement in body weight and reached normal by day 28 (Table 2).

The difference in the blood glucose levels between Day 0 to Day 1 is 6.0mg/dl showing that the test drug has only mild action initially in lowering the blood glucose. While the blood glucose level difference between Day 1 to Day 28 is 142.5mg/dl showing the delayed onset and sustained effect of action.

Test+standard group

In this group it was seen blood glucose levels reduced from 336.7 on day 0 to 327 on day 1(6.65%), 310 on day 3 (15.13%), 293.7 on day 7 (22.13%), 264.8 on day 14 (33.93%), 220.7 on day 21 (47.66%) and 190.7 on day 28 (56.44%) showing that the test drug *Moringaoleifera* (dose of 150mg/kg bw) when combined with Glibenclamide (0.25mg/kg bw) caused reduction in fasting blood glucose levels comparable to the standard drug and showing statistical significance with the diabetic control group (Table 1 and Table 3).

The body weight of this group of rats also decreased following STZ till day 3 after which they showed good improvement in body weight and by the end of day 28 there was increase in body weight of 10% compared to their weight prior to the study (Table 2).

Difference in the blood glucose value in between Day 0 and Day28 in different groups of animals.

- In standard group of rats → -180.0mg/dl.

- In the test group of rats → 142.5mg/dl.
- In the test+ standard group of rats → 146.0mg/dl.

This shows that the test drug when combined with glibenclamide at half the actual dose has brought about good hypoglycemic effect comparable to the standard group.

Table 3: Percentage reduction in blood glucose levels between standard and test groups compared to diabetic control.

Groups	Day 0	Day 1	Day 3	Day 7	Day 14	Day 21	Day 28
Standard group	NA*	4.1 %	10.71 %	18.22 %	33.1 %	52.1 %	61.3 %
Test group	NA*	2.2 %	8.5%	13.3 %	24.25 %	44.29 %	52.9 %
Test+ Std	NA*	6.65 %	15.13 %	22.13 %	33.93 %	47.66 %	56.44 %

*The respective drugs were administered after blood glucose level recording on Day 0, so the data is not being considered

Table 4: Mean change in blood glucose levels at the end of the following weeks among different groups (mg/dl).

Groups	At the end of 1st week	At the end of 2nd week	At the end of 3rd week	At the end of 4th week
Standard	41.3	40.3	66.0	32.4
Test (% <std)	21.1 (49)	24.7 (38)	67.7 (2.5)	29.0 (10.5)
Test+ Std (% <std)	43.0 (4)	28.9 (28)	44.1 (33)	30.0 (2.7)

DISCUSSION

Type 2 Diabetes mellitus is a chronic metabolic disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. The effective and essential means to control raised blood glucose are exercise, diet and weight reduction which are considered to be the prime means of improving glucose homeostasis. However, lifestyle management measures may be insufficient alone or there may be difficulty in patient compliance, rendering the option conventional drug therapies i.e. oral hypoglycemic agents like sulfonylureas, biguanides etc. and making insulin necessary in many patients either alone or with oral hypoglycemic as and when the condition arises.

The presently available drugs are not completely fulfilling the criteria of management of diabetes mellitus. Some drugs may not be helpful to prevent or postpone complications of Diabetes mellitus and some have various adverse effects like hypoglycemia though they are good oral antidiabetic drugs.

Hence in this study an earnest attempt was made to evaluate the effect of ethanolic extract of *Moringa oleifera* leaves on glycemic level and its efficacy has been compared with that of standard oral hypoglycemic drug glibenclamide in Streptozotocin induced diabetic rats which may give an insight for further studies.

The standard group of rats treated with glibenclamide (0.5mg/kg) showed a steady decrease in blood glucose levels from 349.8mg/dl on Day 0 before administration of drug to 169.8 on day 28 thus indicating that the standard drug has a good immediate and also prolonged hypoglycemic action.

Ethanolic extract of Moringa oleifera leaves (300mg/kg) has decreased blood glucose level from 348.8mg/dl on day 0 to 206.3mg/dl on day 28. The progressive hypoglycemic effect with respect to duration of administration and maximum effectiveness of the test drug was after the 1st week, but persistent hypoglycemic activity was continued upto 4 weeks.

The percent reduction of blood glucose level was 52.9% as compared to Glibenclamide group 61.3% (at the end of the study), and has shown similar reduction in mean percent blood glucose level, and it was statistically significant ($p < 0.001$).

The difference in the blood glucose readings for test group between D0 to D28 is 146.0mg/dl and for the standard group the difference in D0 to D28 reading is 180mg/dl, when compared clearly indicating that the test drug is equally potent under the experimental dosage. The above results indicate that ethanolic extract of *Moringa oleifera* leaveshas significant and sustained hypoglycemic activity persisting till last day (28th day).

The group of rats treated with the combination of both glibenclamide and *Moringa oleifera* administered at suboptimal doses also showed reduction in blood glucose level from 336.7mg/dl on day 0 to 190.7mg/dl on day 28 ($P < 0.001$) And the difference in the blood glucose readings between D0 to D28 is also 146.0mg/dl, the percentage reduction in blood glucose level at the end of the experiment being 56.44% indicating that the test drug can be a good adjuvant for the presently available oral hypoglycemic drugs which also helps in reducing their doses thereby decreasing the adverse effect profile and safer treatment of diabetic individuals.

Rats with STZ induced diabetes have reduced body weight, hyperglycemia and hypoinsulinemia because of damaged insulin secreting cells in pancreatic islets.[14] In our study the body weight of STZ induced diabetic albino rats were decreased whereas the body weight gradually increased in Moringa oleifera and glibenclamide treated rats. Hence the ethanolic extract of *Moringaoleifera* has shown more anti diabetic activity by lowering the blood glucose levels in diabetic rats significantly.

Hypoglycemic activity of the leaves of *Moringaoleifera* may probably be due to terpenoid present, which appears to be involved in the stimulation of the ß-cells andthe subsequent secretion of preformed insulin.[15] Result of the preliminary phytochemical screening of *Moringaoleifera* extract has revealed the presence of flavanoids, tannin, anthraquinone, cardiac glycosides, alkaloids, triterpenoids, saponins, and reducing sugars. One ormore of the other chemical constituents of the plante specially flavanoid is also likely to have played a crucialrole in the hypoglycemic action of the plant extract.[16]

Hypoglycemic action may also be due to Alpha-glucosidase inhibitory activity of *Moringaoleifera* or insulin-mimicking action of benzylamine present in *Moringaoleifera* and its stimulation of glucose transport and inhibition of lipolysis.[9,16]

A number of investigators have shown that coumarin, flavanoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids possess hypoglycemic effects in various experimental animal models.[17]

Thus, the present study reestablishes that the ethanolic extract of *Moringaoleifera* leaves is effective in lowering blood sugar levels in streptozotocin induced diabetic albino rats statistically significant and comparable to the standard hypoglycemic drug, glibenclamide.

CONCLUSION

The results have shown that the test compound Moringaoleifera at a dose of 300mg/kg BW has significant and sustained oral hypoglycemic activity in streptozotocin induced diabetic rats compared to diabetic control group being statistically significant (P <0.001) and comparable to the hypoglycemic effect of glibenclamide.

The hypoglycemic effect was shown both when used individually and in combination with glibenclamide used together at suboptimal doses showing that the evaluated plant can be used alone in the management of diabetes at the evaluated dose or can be used as an adjuvant to standard drugs already in use at a lower dose thereby reducing their side effect profile which is a very common encountered issue with many oral antidiabetic drugs.

The study also brought forth the fact that the groups treated with *Moringaoleifera* and combination of *Moringaoleifera* and glibenclamide increased the body weight of rats which was reduced after induction of diabetes with Streptozotocin.

These findings suggest that the hypoglycemic potential of the test compound *Moringaoleifera* is promising and found to be more significant than the diabetic control group. The test drug however seems like a better delayed onset long acting drug with sustained effect and needs to be further evaluated before the compound could be used as an adjuvant to standard hypoglycemic agents for better glycaemic control or as monotherapy in mild to moderate cases.

Further studies are encouraged in this respect along with long term safety studies and clinical trials to add this novel drug to the existing ones for overall management of type2 DM.

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