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Research Article

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Evaluation of hypoglycemic and antihyperglycemic effect of freshly prepared aqueous extract of *Moringa oleifera* leaves in normal and diabetic rabbits

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ABSTRACT

In Indian traditional system of medicine, Moringa oleifera leaves (Moringaceae) is commonly used as healing herb to treat diabetes. This study was undertaken to determine the hypoglycemic effect and antihyperglycemic effect of Moringa oleifera aqueous extract in normal (normoglycemic) and alloxan induced diabetic rabbits respectively. Graded doses of the leaves extract (100, 200 and 300 mg/kg oral) were separately administered to groups of fasted normal and alloxan induced diabetic rabbits. The hypoglycemic and antihyperglycemic effect of the aqueous leaves extract was compared with that of Glibenclamide 0.5mg/kg in fasted normal and alloxan induced diabetic rabbits respectively. Following treatment, Moringa oleifera (100, 200 and 300 mg/kg oral) produced highly significant ($p<0.001$) reduction in blood glucose levels at 2^{nd} hour in fasted normal and alloxan induced diabetic rabbits. But, maximum percentage reduction in blood glucose was seen with 200mg/kg dose when compared to control. The aqueous extract of the leaves of Moringa oleifera possesses hypoglycemic and antihyperglycemic activity in normal and alloxan induced diabetic rabbits respectively.

Key words: Diabetic, Hypoglycemic, Antihyperglycemic, *Moringa oleifera*, Alloxan.

INTRODUCTION

The underlying goal of all diabetes treatment and management is to maintain an adequate blood glucose concentration. Progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in regimen for treatment of this devastating disease. [1] Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The fundamental defect in diabetes mellitus is an absolute or relative lack of biologically active insulin, which results in the impairment of uptake and storage of glucose, reduced glucose utilization for energy purpose. [2] According to WHO projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics world-wide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetic will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with the highest number of diabetics in the world. [3] *Moringa oleifera* is well known for its pharmacological actions and is used for the traditional treatment of diabetes mellitus. [4, 5] With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed. Most of the plants prescribed for Diabetes Mellitus are not edible [6] and therefore, the studies on edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease.

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, or ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. [7] The leaves and pods of *Moringa oleifera* remove all kinds of pain, good vesicant, expectorant, stimulant and abortifacient. The decoction of the leaf is used as a stimulant, analgesic and diuretic. [8] Leaves of *Moringa oleifera* are lopped for fodder [9] and have been used as antiulcer, diuretic, anti-inflammatory and for wound healing.[10, 11, 12, 13] Ethanolic extract of leaves have shown antifungal activity against a number of dermatophytes,[14] whereas methanol extract has a potent CNS depressant action. [15] The aqueous extract of the leaves has been found to possess antifertility activity. [16] The pods are edible, seeds are useful as purgative, antipyretic, cures eye diseases, head complaints and are used in venereal affections. [17]

There are very few studies of hypoglycemic and antihyperglycemic effect of *Moringa oleifera* leaves in rabbits. So, this study was carried out to critically evaluate the hypoglycemic and antihyperglycemic effect of aqueous leaves extract of *Moringa oleifera* in normoglycemic and alloxan induced diabetic rabbits respectively.

EXPERIMENTAL SECTION

Plant material: *Moringa oleifera* leaves was collected from the local market and were authenticated by Professor and Head, Department of Botany, Government Degree College, Khammam.

Extract preparation: The leaves of *Moringa oleifera* were collected and dried under shade and ground into powder. Aqueous extract of *Moringa oleifera* leaves was done in the department of Pharmacology, Mamata Medical College, Khammam using continuous Soxhlet Extraction or Soxhelation. [18]

Acute toxicity study: The LD_{50} was found to be more than 5000mg/kg per oral in acute toxicity testing. [19] Based on this, three doses of 100, 200 and 300mg/kg were selected.

Chemicals used: All chemicals and drugs used were obtained commercially and of analytical grade. Alloxan monohydrate (Sigma chemicals St. Louis, USA) and Tab Glibenclamide 5mg (Aventis pharm ltd, Ankleswar)

Animals used:
All the animals included in the study were procured from animal house of Mamata Medical College, Khammam. Laboratory breed of New Zealand white rabbits of either sex weighing 2-2.5kg were used for the study. The animals were maintained under standard laboratory conditions at 25°c, commercial pellet diet with water ad libitum & normal photo period (12hr dark/12hr light).

Approval was taken from the Institutional Animal Ethics Committee for the study.

The animals were fasted for 12-18 h with free access to water prior to the administration of the extract. After 12-18 h, the blood glucose levels was measured using the glucose-oxidase principle and the fasting blood glucose greater than 50 mg/dl was included in the study for the normoglycemic group and fasting blood glucose level greater than 150 mg/dl for the diabetic group.

The normoglycemic rabbits were randomly assigned into five groups (1-5) of five rabbits (n = 5) each as follows, namely:
Group 1: Normal, Control (Normal saline, 5 ml/kg bodyweight orally)
Group 2: Normal, Standard (Glibenclamide 0.5mg/kg dissolved in distilled water orally)
Group 3: Normal, test compound (Moringa oleifera 100 mg/kg extract orally)
Group 4: Normal, test compound (Moringa oleifera 200 mg/kg extract orally)
Group 5: Normal, test compound (Moringa oleifera 300 mg/kg extract orally)

The overnight fasted animals were infused via the ear vein with 150 mg/kg alloxan monohydrate for 10min after taking their fasting blood glucose values. Then they were allowed to have free access to food and water. After 1 week of alloxan induction the animals with fasting blood glucose levels between 300-350mg/dl were included in the

study. Diabetic rabbits were randomly assigned into five groups (1-5) of five rabbits (n = 5) each as follows, namely:

Group 1: Diabetic, Control (were given normal saline, 5 ml/kg bodyweight orally)
Group 2: Diabetic, Standard (Glibenclamide 0.5mg/kg dissolved in distilled water orally)
Group 3: Diabetic, test compound (Moringa oleifera 100 mg/kg extract orally)
Group 4: Diabetic, test compound (Moringa oleifera 200 mg/kg extract orally)
Group 5: Diabetic, test compound (Moringa oleifera 300 mg/kg extract orally)

Determination of blood glucose levels: Determination of the blood glucose levels was done by the glucose-oxidase principle [20] at interval of 0, 1, 2, and 4 hours by puncturing marginal vein of the rabbit's ear. Blood glucose was estimated by using glucometer (Accu-Chek sensor from Roche Diagnostic Corporation) and results were expressed as mg/dl. [21]

Statistical analysis: Blood glucose levels were expressed in mg/dl as mean±SEM. The statistical analysis of data was done using one way analysis of variance (ANOVA), followed by Dunnett's test using the software "PRIMER OF BIOSTATISTICS". P value less than 0.05 was considered to be significant.

RESULTS

Effect on blood glucose level of normoglycemic rabbits: Table 1 describes the hypoglycemic effect of graded doses of aqueous extract of *Moringa oleifera* leaves on fasting blood glucose level of normal rabbits**.** 100mg/kg showed significant decrease in blood glucose only at 1h and 2h (P<0.05). 200mg/kg significantly decreased blood glucose at 1h, 2h (P<0.001) and 4h (P<0.05). At 1h, 2h and 4h, 300 mg/kg of the extract showed significant (P<0.05) decrease in the blood glucose levels. Maximum reduction in blood glucose level was 14.01 % at 2h with 200mg/kg, whereas it was 10.03 and 10.50% with 100 and 300mg/kg respectively and 16.40% with Glibenclamide as compared to control.

Table 1: Effect of aqueous leaves extract of *Moringa oleifera* on blood glucose level of Normoglycemic rabbits

Treatment	Blood glucose levels (mg/dl)			
	0 h	1 h	2 h	4 h
Control(Normal saline)	129.60±1.02	127.40±1.28	125.60±1.03	128.0±0.89
Glibenclamide 0.5mg/kg	129.40±0.81	109.40±0.87**	105.0±1.30**	98.60±1.50**
*Moringa oleifera*100mg/kg	126.40±2.13	116.80±2.17*	113.0±3.13*	121.20±2.87
*Moringa oleifera*200mg/kg	138.40±5.08	118.0±5.17**	108.0±1.37**	115.60±2.92*
*Moringa oleifera*300mg/kg	129.60±1.02	127.40±1.28*	112.40±0.87*	116.0±1.58*

*Values are mean±SEM, n=5, * P<0.05, **P<0.001*

Table 2: Effect of aqueous leaves extract of *Moringa oleifera* on blood glucose level of Alloxan induced diabetic rabbits

Treatment	Blood glucose levels (mg/dl)			
	0 h	1 h	2 h	4 h
Control(Normal saline)	331.80±1.93	328.0±1.61	324.80±2.29	329.60±1.69
Glibenclamide 0.5mg/kg	331.80±1.93	275.20±8.44**	265.60±4.90**	257.0±3.96**
*Moringa oleifera*100mg/kg	308.20±9.01	285.60±7.38**	279.20±6.95**	294.0±7.03*
*Moringa oleifera*200mg/kg	324.60±10.30	282.40±8.70**	275.40±8.28**	307.60±7.62*
*Moringa oleifera*300mg/kg	313.40±11.26	286.60±10.98*	278.60±11.4*	296.60±9.21*

*Values are mean±SEM, n=5, * P<0.05, **P<0.001*

Effect on blood glucose level of hyperglycemic rabbits: Table 2 demonstrates the effect of graded doses of aqueous extract of *Moringa oleifera* leaves on blood glucose level of hyperglycemic rabbits. There was significant decrease in the blood glucose levels at 1h, 2h (P<0.001) and 4h (P<0.05) with 100 and 200mg/kg. With 300mg/kg

there was a significant decrease (P<0.05) in the blood glucose levels at 1h, 2h and 4h. Maximum reduction in blood glucose level was 15.20% at 2h with 200mg/kg, whereas it was 14.03 and 14.22 % with 100 and 300mg/kg respectively and 18.22% with Glibenclamide as compared to control.

DISCUSSION

Moringa oleifera leaves have been shown to have glucose lowering effect in studies on normoglycemic and hyperglycemic rats. [22, 23, 24] Our study showed hypoglycemic and antihyperglycemic activity of aqueous extract of *Moringa oleifera* leaves in normal and alloxan induced diabetic rabbits respectively. Doses of 100, 200 and 300 mg/kg of the extract showed significant decrease in the blood glucose levels at 2 h both in normal ($p<0.001$) and diabetic rabbits ($p<0.001$), but maximum reduction was seen with 200mg/kg. At 2 h blood glucose was decreased by 14.01 and 10.50%, in normal and 15.20 and 14.22% in diabetic rabbits treated with 200mg/kg and 300mg/kg respectively. Such a phenomenon of less hypoglycemic response at higher doses is common with indigenous plants and has already been observed in Psidium guajava [25], Trichosanthes dioica [26], Cynodon dactylon [27, 28] and Cinnamomum tamala.[29]

Phytochemical screening of *Moringa oleifera* extract revealed the presence of flavinoids, tannin, anthraquinone, cardiac glycosides alkaloids, triterpenoids, saponins, and reducing sugars. [30]

A number of investigators have shown that coumarin, flavonoid, terpenoid and a host of other secondary plant metabolites including arginine and glutamic acids posses hypoglycemic effects in various experimental animals model. [31, 32]

Hypoglycemic and antihyperglycemic activity of the leaves of *Moringa oleifera* may be probably due to the presence of terpenoids, which appears to be involved in the stimulation of the β-cells and the subsequent secretion of preformed insulin. [33] One or more of the other chemical constituents of the plant especially flavonoid is also likely to have played a crucial role in the hypoglycemic action of the plant extract. Further studies are required to isolate and characterized the active components of the extract of this plant.

CONCLUSION

The present study showed that aqueous leaves extract of *Moringa oleifera* possessed hypoglycemic and antihyperglycemic properties in normal and alloxan induced diabetic rabbits respectively, which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are currently under way to isolate and characterized the active components of the crude extract of this plant.

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