

Research Article

Effects of *Moringa oleifera* Leaf Extract on Blood Sugar Parameters in Type2 Diabetic Patients: An Observational Comparative Study

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Abstract: *Moringa oleifera* is one of the world's most useful trees. It has been claimed to possess antihyperglycemic properties in Indian traditional system of medicine for past few decades. Phytoconstituents obtained from *Moringa oleifera* have been reported to possess antihyperglycemic effects amongst which are glucosinolates, isothiocyanates, phenolic compounds and β -sitosterol. Isothiocyanates have been documented to induce insulin secretion and glucose homeostasis. Plant products are safer and effective alternative remedies with low costs, limited adverse consequences and easy accessibility. In the light of above information, the present study was conducted to emphasize the antihyperglycemic potential of *Moringa oleifera* in leaf extract capsules. A total of 90 participants were enrolled in this study and divided into three groups amongst which 30 healthy nondiabetic individuals were included. Assessment of blood glucose parameters Fasting blood sugar (FBS), Post prandial blood sugar (PPBS) and HbA_{1c} were done at baseline, 12 weeks and 24 weeks. At the end of 12 and 24 weeks *Moringa oleifera* capsules at a dose of 500mg BD were found to reduce blood glucose parameters both solely and also in combination with Metformin significantly. This emphasizes the antihyperglycemic properties of *Moringa oleifera* capsules.

Keywords: *Moringa oleifera* leaf extract, Isothiocyanates, antihyperglycemic, type2DM.

INTRODUCTION

Diabetes is known since ancient past. It has also been mentioned in the writings of ancient civilizations, especially Egypt, Arabia, India, China and Asia Minor.

An estimation was made that in the year 2000, that there were 171 million diabetic people in the world. India is the diabetes capital of the world with 41 million Indians having diabetes; every fifth diabetic in the world is an Indian. It also leads in prevalence of metabolic syndrome as well as obesity. 20 million Indians are either obese or abdominally obese with children being the prime targets and by 2025, the expected number is 68 million. The estimation is expected to increase to reach 366 million diabetic people by 2030 (Ali, H. *et al.*, 2006).

Diabetes mellitus is defined as a disease in which the body is unable to use and store glucose, properly. An abnormality of carbohydrate metabolism linked to either low blood insulin level or insensitivity of target organs to insulin leads to diabetes (WHO. 2006; Maiti, R. *et al.*, 2004).

Up to 2,50,000 children in developing countries under the age of 14 years have type 1 diabetes; around 38,000 of these children are in Africa. Extracts of various plant materials capable of decreasing blood sugar have been tested in experimental animal models and many clinical studies have also been done (Kavishankar, G. B. *et al.*, 2011; Das, A.K., & Shah, S. 2011).

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According to WHO, 70-80% of the world's population in developing countries rely on herbal medicines for prevention and cure of diseases (Ekor 2014) and 25% of the synthesized drugs are manufactured from medicinal plants (Pan *et al.*, 2013). The present antihyperglycemic agents possess many adverse consequences (Ganguly, R., Guha, D. 2008). Great consideration has been aimed at bringing forward antidiabetic potential of therapeutic foliage plus their herbal formulations in the management of diabetes (Mbikay, M. 2012; Jaiswal, D. *et al.*, 2009).

Moringa oleifera has been attracting attention for the past decade as an effective agent towards chronic ailments. The genus *Moringa* is one of the genera found in the Moringaceae family. Commonly called as 'drumstick' or 'horseradish' tree. *Moringa* genus comprises of 13 species that are distributed throughout Southwest Asia, Southwest Africa, Northeast Africa and Madagascar. *Moringa* is indigenous to the Himalayan foothills (Northern India, Pakistan and Nepal) (*Moringa oleifera* for Diabetes). Later the species has been cultivated throughout the world specifically in Asia, Florida, The Caribbean and Pacific Islands (Fahey, 2005).

This soft wood tree measures about 12 metre in height. It can grow in any tropical and subtropical climates with peculiar environmental features. It is able to resist dry to moist temperatures with an annual precipitation of 760-2500mm and temperature between 18-28°C. An altitude of 2000m can be reached. The *Moringa* genus are categorised into three groups depending on the type of trunk they have (Olson and Rosell 2006). *Moringa stenopetala*, *Moringa drouhardii*, *Moringa ovalifolia* have water storing trunks while *Moringa oleifera* has slender trunk. *Moringa* species are resistant to drought and grows fast without much care (Paliwal, R., & Sharma, V. 2011).

Phytochemistry

Moringa species contains various phytoconstituents namely alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates (Glucosides), flavonoids and terpenes (Animashaun, J. 2013). The huge diversity in phytoconstituents contributes to its numerous pharmacological actions and uses (Teixeira, E. M. B. *et al.*, 2014).

Only few species have been explored in this genus including *Moringa oleifera* and majority of the studies focuses on the leaves. Out of all the constituents Glucosinolates are the most therapeutically active component present in leaves. Hence most of the drug preparations of *Moringa* employ leaf extract for the production of active metabolites in therapeutics (Förster, N. *et al.*, 2015; Dinkova-Kostova, A.T., & Kostov, R.V. 2012).

Moringa oleifera has been claimed to possess antihyperglycemic effect in Indian traditional system of medicine (Divi, S. M. *et al.*, 2012; Padayachee, B., & Baijnath, H. 2012).

Moringa oleifera as a rich source of ascorbic acid is postulated to stimulate insulin secretion. Certain nutrients like vitamins B1, B2, B12, pantothenic acid, vitamin C, protein and potassium- along with small frequent meals containing some carbohydrate can actually stimulate production of insulin within the body (Sharma, V. *et al.*, 2011).

Moringa species are rich in glucosinolates. These are secondary plant metabolites that are mostly isolated from Brassicaceae family (e.g Broccoli, *Moringa* spp., Cabbage, Sprouts, watercress, garden cress, Cauliflower). Broccoli is a rich source of glucosinolate Glucoraphanin (22mg/100g) and Glucobrassicin (17mg/100g). The most abundant glucosinolate present in this species is 4-o-(α -L-rhamnopyranosyloxy)-benzyl glucosinolate, also called as 'Glucomoringin' (GMG) [18]. Myrosinase and GLS reside in different compartments but they come in contact upon tissue damage. E.g. chewing, cutting resulting in rapid hydrolysis (Navarro, S. L., Li, F., & Lampe, J. W. 2011).

A study in the Virudhunagar district of Tamil Nadu reports *Moringa* amongst the species utilized by traditional Siddha healers [20]. Many compounds isolated from *Moringa oleifera* leaves may be involved in the glucose homeostasis. Amongst them, isothiocyanates seems to reduce insulin resistance and hepatic gluconeogenesis [21]. In a study of Ndong *et al.*, male spontaneously diabetic Goto-Kakizaki (GK) rats and non-diabetic male Wistar rats received a single dose of glucose solution and a dose *Moringa oleifera* leaves (2 g/kg BW and 200 mg/kg BW, respectively), whereas control groups of both animals only received a single dose of glucose solution. Blood glucose concentration was measured at 0, 10, 20, 30, 45, 60, 90 and 120 min. Results from OGTT shows that *Moringa oleifera* significantly decreased blood glucose at 20, 30, 45, and 60min in GK rats compared to the control and at 10, 30 and 45 min. Moreover, in GK rats, the treatment with *Moringa oleifera* leaves reduced Area under curve (AUC) values by 23%, whereas it did not significantly affect these values in control rats. These results suggests that *Moringa oleifera* has a glucose intolerance ameliorating effect in both GK and Wistar rats, with a greater action in diabetic than in normoglycemic rats (Ndong, M. *et al.*, 2007).

Natural resources are nowadays considered as potent candidates for drug discoveries. They will play a pivotal role in drug development programs in upcoming years. Medicinal herbs, a rich mine for bioactive chemicals are markedly free from undesirable side

effects and have powerful pharmacological actions. The secondary metabolites could act as lead compounds for the discovery of different new classes of possibly potent and safe antidiabetic agents. Attention must be brought towards the identification of the typical modes of action of their extracts and the isolated pure compounds (Talreja, T. 2010).

Moringa diversifies in many features and high morphological variability which becomes a resource for the conservation of *Moringa oleifera* germplasm. However, some questions still remain unanswered, *i.e.*

1. Many *in vitro* and *in vivo* studies in animals widely confirms the pharmacological properties but only few evidences on human beings are available .
2. On the other hand, the leaves extract contain oxalates and phytates in large amount that could limit the intestinal absorption of minerals.
3. Further studies aimed to confirm the pharmacological effects of moringa on human beings and, at the same time, ensuring its safety on human health for chronic or long-term use should be probed.
4. Through this study we aim at supporting the antihyperglycemic effects of Coeringa capsules that contains *Moringa oleifera* leaf extract.

MATERIALS AND METHODS

Workplace

This observational comparative follow up study was conducted in the Department of Pharmacology & Therapeutics, Rajendra Institute of Medical Sciences (RIMS), Ranchi amongst the patients attending Medicine outdoor department.

Ethical Consideration

The approval was taken prior from the 'Institutional Ethics Committee' of Rajendra Institute of Medical Sciences (RIMS), Ranchi. Case Record form and Informed Consent form were framed prior to including patients in the study.

A written informed consent was obtained his/her signature. Left thumb impression was taken from illiterate patients. All these above procedures were done in the presence of an appropriate and unbiased witness.

Study Design

An Observational Comparative Follow up Study.

Study Duration

The study was conducted from June 2016 till July 2018. Every participant was followed up for 24weeks in totality following their selection and screening.

Inclusion Criteria

Patients having Fasting blood glucose (FBG) ranging between 126-200mg/dl whose age ranged between 30-60yrs of either sex were included in the study. Newly diagnosed or untreated diabetic cases having creatinine clearance <60 and patients already taking *Moringa oleifera* capsules with or without combination with other drugs namely Metformin were also included in this study.

Exclusion criteria

pregnant and lactating women, patients with Cardiovascular diseases, hepatobiliary pancreatic diseases, renovascular diseases, patients using other oral hypoglycaemics like Sulfonylureas, Meglitinides etc. or insulin, history of Stroke, TIA (Transient ischemic attack), MI (Myocardial Infarction) in past 6 months and patients who were already a participant in another study were excluded from the study.

Study methodology

Patients were screened for diabetes for about 1-2weeks and amongst them who qualified were comprehended about the study in a simple non-technical language that was easy to understand by the patient. Participant's consent was taken in writing (Literate) and Left thumb impression for illiterate using the Informed consent form in the presence of an appropriate unbiased witness. The participants were randomly allocated their respective groups accordingly as per OPD visit and followed up. Behavioural counselling was also done regarding their medical condition. Every participant was followed up for 6 months commencing from their screening and group allocation. They were counselled to visit the centre at 12 weeks and at the end of 24 weeks. The GROUP allocation was allocated as follows:

Group A: Individuals who are not taking any antidiabetic drugs. E.g Individuals on antibiotics etc.

Group B: Patients who were prescribed *Moringa oleifera* capsules (500mg BD).

Group C: Patients who were prescribed Moringa (500mg BD)+ Metformin(500mg BD).

Detailed history and thorough clinical examination was done for all the participants. The participant's biochemical parameters like Fasting blood sugar (FBS), Post prandial blood sugar (PPBS), Glycosylated haemoglobin (HbA1c) were evaluated at the end of 12 and 24weeks. The blood sample of every participant was taken and sent to the laboratory for further investigations. Case record forms were maintained and filled in all subsequent visits.

Method for Collection of Blood & Glucose Estimation

The participant is made to sit on a chair after washing of hands and drying. The glucometer is turned on and a test strip is prepared. The lancing device

(usually in the shape of pen) is held and the sides of the finger is pricked after choosing a spot. A drop of blood is put over the test strip which has already been inserted in the glucometer beforehand. Within 5-10secs the monitor displays the value. The value is recorded and done in a similar way for other participants also. PPBS is recorded in similar manner 2hrs after food. The method used to estimate the blood glucose was Glucose oxidase method from the capillary blood. For HbA1C values laboratory methods were used.

Diagnostic Evaluation

Repeated measurements of FBS, PPBS, HbA1c were taken accompanied by detailed family history, clinical history, physical examination and laboratory investigations.

Clinical history

Thorough family history, chief complaints, history of present illness, past history, personal history and treatment history was taken and recorded. Menstrual history was taken from females.

Follow up schedule

Follow ups were scheduled at 12weeks and 24weeks, commencing from the screening procedure and allocation of groups.

Assessment of any untoward effects

During the screening visits every participant undergoes a physical examination and medical history. Any adverse event or untoward effects were collected and recorded in the upcoming follow up visits.

Statistical analysis

All the participants at the end of 24weeks were evaluated by summing up all the data available. The data were expressed as Mean and Standard deviation (SD). P value <.05 was considered as statistically significant. Data were entered in Microsoft excel 2010 and Data evaluated in IBM SPSS 20.0 version. For Intragroup comparisons Independent sample t test was used. For Intergroup comparisons Leveny' s test was used.

RESULTS

Data were expressed as Mean, Standard deviation and as % wherever required. 'p value' <0.05 was considered as statistically significant. No. of patients fulfilling the Inclusion criteria were 90. No. of males were 59 and 31 females. 9 patients withdrew from the study due to noncompliance. No. of patients in Group A were 26, Group B were 28 and in Group C were 27.

Table.1

Mean FBS Values (mg/dl) of Groub 'a', 'b', 'c' at base line, 12 & 24 Weeks			
Groups	FBS (mg/dl) BL	FBS (mg/dl) at 12 Weeks	FBS (mg/dl) at 24 Weeks
	± SD	± SD	± SD
Control (Group-a)	79.62 ± 6.84	79.54 ± 9.10	82.69 ± 6.49
Moringa (Group-b)	159.52 ± 24.16	153.38 ± 24.89	147.21 ± 24.80
Moringa + Metformin (Group-c)	171.93 ± 17.36	165.67 ± 17.81	159.41 ± 18.682

Table.2

Mean PPBS values (mg/dl) of Groub 'a', 'b', 'c' at Base line, 12 & 24 weeks			
Groups	PPBS (mg/dl) BL	PPBS (mg/dl) at 12 Weeks	PPBS (mg/dl) at 24 Weeks
	± SD	± SD	± SD
Control (group-a)	126.15 ± 18.09	129.04 ± 16.43	131.92 ± 14.77
Moringa (group-b)	196.41 ± 25.09	186.69 ± 24.47	180.03 ± 24.10
Moringa + Metformin (group-c)	208.41 ± 19.99	199.74 ± 19.33	190.96 ± 19.84

Table.3

Mean Hba1c values (mg/dl) of Group 'a', 'b', 'c' at base line, 12 & 24 weeks			
Groups	HbA1c (mg/dl) BL	HbA1c (mg/dl) at 12 Weeks	HbA1c (mg/dl) at 24 Weeks
	± SD	± SD	± SD
Control (group-a)	5.192 ± 0.46	5.25 ± 0.34	5.26 ± 0.28
Moringa (group-b)	7.32 ± 0.89	7.06 ± 0.90	6.72 ± 0.92
Moringa + Metformin (group-c)	7.60 ± 0.89	7.43 ± 0.90	5.68 ± 0.88

DISCUSSION

The present study was conducted in RIMS, Ranchi from June '16 to July '18 to evaluate the antihyperglycemic effects of *Moringa oleifera* leaves extract. Similar kind of study was done by Rutchaporn Taweerutchana *et al.*, which was a Randomized Placebo Controlled study, 28th Nov'17.

Effects of *Moringa oleifera* (Group B) on blood glucose levels

In this study, participants taking moringa, the FBS mean value reduction at the end of 12 and 24 weeks was statistically significant, p value was .001 and .004 respectively. Mean PPBS reduction at the end of 24 weeks was 8% however it was statistically insignificant. Mean HbA1c reduction at the end of 24weeks was 8% although it was statistically insignificant. The mean value reduction in FBS, PPBS & HbA1c might be attributable to the phytoconstituent Isothiocyanates present in *Moringa oleifera* leaves extract which stimulates Akt (Ak strain transforming) pathway that has a major role in insulin signalling and thus exhibits antidiabetic potential in type 2 DM.

Similar kind of study was performed by Rutchaporn Taweerutchana *et al.*, in Department of Medicine, Mahidol university, Siriraj hospital, Thailand which revealed decrease in FPG & HbA1c Mean values in both MO leaves group and control group but p values (.44 for FBS,.37 for HbA1c respectively) for them were statistically insignificant.

Cochrane review 2012 states about a 6 month follow up study done in newly diagnosed type2 DM patients taking Moringa leaves extract. Patients taking *Moringa oleifera* leaves extract had significant decrease of HbA1c as compared to control group.

Widespread claims have been made at the Johns hopkins university regarding the effectiveness of *Moringa oleifera* preparations. A plethora of literature adjoins to its curative tendencies.

In a study performed by Ndong *et al.*, (2007), Goto-kakizaki (GK) Wistar rats were induced Diabetes mellitus. GK rats have tendency to develop early glucose tolerance (Bisbis *et al.*,1993; Abdel-Halim *et al.*,1995). Treatment with *M. oleifera* leaf powder resulted in lower glycemic response in GK and control rats. In GK rats, the treatment reduced Area under curve values by 23% (p value<.05) whereas values in control rats were not affected.

Effects of *Moringa*+Metformin (Group C) on the blood glucose levels

In the present study, mean FBS reduction at the end of 24weeks was highly statistically significant (p value .000). Mean PPBS reduction at the end of 24weeks was highly statistically significant (p value

.000). Mean HbA1c reduction at the end of 24weeks was highly significant (p value .000). This effect might have occurred due to metformin, which efficiently reduces Blood sugar level through AMP activated protein kinase (AMPK) mechanism (Shaw *et al.*, 2005).

Similar kind of study was sponsored by Titilayo O Fakeye, University of Ibadan (June 16, 2017). In a study done by Graham Rena *et al.*, March'17 the antidiabetic potential of Metformin was demonstrated through AMPK mechanism.

CONCLUSION

Moringa oleifera capsules that were used in this study is promising as an antihyperglycemic drug. When Moringa was used along with metformin potentiating effect of Moringa capsules was observed. *Moringa oleifera* did not pertain to significant side effects which makes it a safe drug. More researches on *Moringa oleifera* should be encouraged.

It is essential to exploit the phytoconstituents of each part of *Moringa oleifera* plant for chronic diseases. More randomized double blind trials be employed to substantiate the primary mechanism of action of Moringa as an antihyperglycemic drug.

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