Impact of *Moringa oleifera* (MO) on diabetic and Healthy patient's: In Vivo Study

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Abstract: There hasn't been much research done on Moringa oleifera (MO) leaf powder's hypoglycemic impact in people. We evaluated the chemical makeup and dietary acceptability of MO leaf powder made from several patients in the Ambala region (Northern Haryana). We next randomly gave a regular meal supplemented with 25 g of MO leaf powder (MOR20) or not (control meal, CM) to 18 diabetics and 10 healthy volunteers on two separate days to assess its impact on postprandial glucose response. Capillary glycaemia was assessed just before the meal and then every 30 minutes for three hours. When using MOR20 instead of CNT, the postprandial glucose response peaked sooner in the diabetic patients and had smaller increments at 60, 120, and 130 minutes. Compared to the prior one, the mean glycemic meal reaction with MOR20 was lower. There were no differences among the healthy participants. As a result, MO leaf powder may be a natural medication that lowers blood sugar. Lower dosages must to be considered, nevertheless, considering the 25 g MO meal's poor taste acceptance. Furthermore, studies assessing MO leaf powder's long-term effects on glycaemia should also show its hypoglycemic effects.

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Introduction: Native to the Indian subcontinent, Moringa oleifera (MO) is a multipurpose plant that has spread throughout tropical and subtropical regions of the world due to its capacity to thrive in both hot, dry, and humid environments as well as less fertile soils that are frequently impacted by drought [1,2]. Many African and Asian people eat the plant's leaves raw or powdered [3], since they are high in protein and vitamins, including beta-carotene, a precursor to vitamin A, minerals, and bioactive chemicals [4]. Because of their chemical-nutritional properties, the leaves are suitable candidates to be combined with regional foods to enhance the diets of people in poor nations and lower the risk of malnutrition. It must be acknowledged, nevertheless, that there have been few human clinical trials testing MO's efficacy in treating malnutrition, and the findings have been mixed [5–7]. This could rely, at least in part, on the various amounts of leaf powder employed in the research, which range from 14 to 30 g daily for one to six months. In fact, a dose of a supplement that is too low could not be enough to have the desired effect, while a dose that is too high might cause the patient to experience undesirable side effects, making it unsuitable for usage outside of a research setting. The bitter taste of the leaves might sometimes detract from the enjoyment of culinary preparations. According to recent research, the more Moringa there is in a dish, the less acceptable it is [8]. Therefore, before beginning a study, it is crucial to determine the dosage of MO leaf powder.

Apart from its application in human nourishment, MO leaves are also utilized as a medicinal plant in many underdeveloped nations' traditional medicine to treat a variety of illnesses, including as diabetes and hyperglycemia [1,3]. MO leaf and its extracts have been demonstrated in several investigations in both diabetic and non-diabetic animal models to increase glucose tolerance and lower plasma glucose levels [9–15]. Fiber and a variety of secondary metabolites, including glucosinolates, isothiocyanates, flavonoids, and phenolic acids, some of which have the ability to block amylase, have been primarily linked to this hypoglycemic action in leaves [16]. This would decrease the rate of intestinal absorption of glucose and intestinal digestion of starch, lowering the postprandial glycemic peak and, therefore, the risk of acquiring diabetes. It would also improve the control of glycaemia in those with diabetes. This technique has been shown to work in healthy subjects when Phaseolus vulgaris extract is added to starchy meals [12]. In diabetic patients, however, the same mechanism is now employed when acarbose is administered. Unfortunately, there are relatively few and conflicting research showing that MO leaves have hypoglycemic effects on people [7,10]. In the Saharawi refugee camps in southwest Algeria, where they have been residing since 1975, refugees from Western Sahara have been growing MO trees for a number of years due to the agronomic qualities of the MO plant and the nutritional value of MOleaves. For their survival, the Saharawi people do, in fact, rely entirely on food rations and nutritional supplements supplied by the international community. Cereals, legumes, sugar, oil, and blended foods are all included in these monthly rations [10]. On the other hand, virtually little fresh fruit and vegetables are consumed. Diabetes is the most common health issue among the Saharawi people [15], followed by obesity and stunting in children [5], the latter of which is brought on by a low intake and quality of proteins. This is because of their dietary pattern, which is characterized by a high intake of carbohydrates, particularly sugars and complex carbohydrates with a high glycemic index, such as refined rice, white bread, and pasta.

. In this regard, using MO leaves in conjunction with regional food preparations may help improve blood glucose control in diabetic subjects and increase the intake of nutrients that are typically deficient in these people's diets. It may also lower the glycemic index of meals, which is thought to lower the risk of developing diabetes. Thus, the objectives of this study were to: • Assess the sensory acceptability of a local food preparation that included MO leaf powder; and * Assess the impact of the locally produced, at-home MO leaf powder on the postprandial glucose response in both healthy subjects and diabetic patients in the Ambala region.

2. Materials and Methods

Research Design There were four steps in the current investigation.

Step 1: Acceptability test to assess the overall acceptability and sensory qualities of a conventional meal that has 25 g of MO leaf powder added.

Step 2: Glycemic response test to assess the effects of a standard meal supplemented with 25 g of MO leaf powder on the postprandial glucose response in participants with and without diabetes.

Step 4: The powdered leaves that were sold commercially were used.

2.1. Participants Volunteers from the Ambala region, who reside in Northern Haryana, were chosen in January 2024 from among both non-diabetic and diabetic participants. Dietary supplements were used as the foundation for recruitment. They had to be of normal weight, appear to be in excellent health, and not be using any long-term drugs. Among the patients, the diabetics were chosen. Participants in the trial required to have been diagnosed with type 2 diabetes for a minimum of one year, be free of advanced retinopathy, terminal renal failure, active diabetic ulcers, amputations, and heart failure, and be receiving only oral hypoglycemic medications instead of insulin therapy.

2.2. Preparing Meals A traditional meal was a diet rich in protein. Fenugreek seeds, the traditional northern Haryana dish, and all the other ingredients were purchased from local vendors. After 15 minutes of sautéing each food item with onions, carrots, garlic, and tomatoes in sunflower oil, water was added, and the pot was left to simmer for two hours. Each individual received two portions of the meals; one portion was administered without MO leaf powder (control meal, CM) and the second was augmented with 25 g of MO leaf powder (MOR20). We chose the 25 g dosage because it falls within the range documented in the literature and because, based on our prior unofficial experience; it was both easily ingested in the event of chronic usage and acceptable from a sensory perspective.

Test of Acceptability On the day of the test, the diabetic and healthy volunteers were seated in a cozy area at the Amabal Diabetic patients' dispensary. A number of testing cabins were then put up in an adjacent room, and they were accommodated one by one. After being assigned, each participant was instructed to eat 10 g of MOR20 and CM. The volunteers were instructed to test the samples in the order that they were provided, which was a computer-generated randomized order. The individuals sipped water after assessing and ingesting the first sample, and then tasted the second. Using a 12-cm linear scale with the anchors "dislike extremely" on the left and "like extremely" on the right, the participants were asked to rate the sample's color, taste, texture, and general acceptability. Because the addition of MO leaf powder rendered the testing meal green and therefore immediately identifiable, neither the participants nor the investigators were blinded during the test.

2.3 Test of Glycemic Response Each patient was given two identical-looking meals on two distinct days in a computer-generated random order, with the exception of the inclusion of a specific quantity of 25 g of MO. The volunteers were instructed to have their final meal before the experiment by 8 or 9 p.m. on the evening before each one. Based on each subject's dietary patterns, the evening meal required to consist of the typical meals in the typical amounts. Water was the only beverage permitted after 9 p.m. The diabetic participants were told to take their final hypoglycemic drugs by midnight on the day before the research. The next day at 9:30 a.m., the individuals had their finger pricks for fasting capillary whole blood taken, and a GlucoMen glucometer equipped with glucose-oxidase-based test strips was used to assess their glucose levels.

Each individual was given the test meal right away, and the capillary blood glucose level was assessed as previously mentioned at 30, 60, 90, 120, 150, and 180 minutes after the meal started. The nurse at the dispensary kept an eye on each subject as they ate to make sure they finished the meal in the allotted twenty minutes. Furthermore, in this instance, the doctors who took the blood samples were not told which meal the individuals had eaten, but the participants were not blindfolded.

Analysis of Statistics The normality of continuous variables was examined. While mean and standard error were used to show the data on the chemical and sensory assessments as well as the postprandial glucose response, mean and standard deviation were used to represent the basic characteristics of the recruited participants (age and BMI). A paired T-Test was used to examine the sensory attributes and postprandial glycemic responses to MOR20 and CM. The maximal glucose concentration, the duration of maximal peak, the mean glucose response, and capillary glycaemia expressed as concentration and change from the baseline at different time intervals were used to evaluate the postprandial glucose response. The latter is the mean of the glycemic readings for a particular person at every OGTT time point. When the p-value was less than 0.05, it was deemed statistically significant. Microsoft Excel's was used for statistical analysis.

3.0 Results

Acceptability Test

Participants in the acceptance test assessed the test meal's color, flavor, texture, and general acceptability both with and without the addition of 25 g of MO leaf powder. The findings are displayed in Table 1, where it is evident that the addition of leaf powder often resulted in lower ratings for color and flavor but not texture. Additionally, there was a non-statistically significant decrease in overall acceptability (p = 0.055).

Table 1: Sensory and overall acceptability of the meals supplemented and not supplemented, with MO leaf powder

Sensory	**MOR20**	**CM**	***p*-Value**
Color	4.5 ± 0.5	5.6 ± 0.5	0.003
Taste	4.4 ± 0.4	5.4 ± 0.4	0.024
Texture	4.8 ± 0.6	5.8 ± 0.6	0.064
Acceptability	4.6 ± 0.5	5.4 ± 0.5	0.055

Values are mean and standard errors. Values are scores obtained using a 12-cm linear scale, with anchors of “dislike extremely” on the left and “like extremely” on the right. Means were compared using the *T*-Test on Excel calculation.

The baseline blood glucose levels in healthy participants were comparable on both occasions. Following MOR20 and CM, postprandial blood glucose peaked at comparable periods and concentrations (50 ± 5 min vs. 50 ± 10 min, p = 0.719; 119 ± 5 mg/dL vs. 135 ± 7 mg/dL, p = 0.067). At no time point were there any appreciable variations in absolute or incremental glucose concentrations. There was no difference in the mean meal glycemic response with MOR20 (101 ± 4 mg/dL) and CM (105 ± 4 mg/dL, p = 0.245). During the two trials, the baseline blood glucose levels of the diabetic individuals were comparable. Compared to CM, MOR20 caused the postprandial glucose response to peak earlier and at lower amounts. Blood glucose levels were consistently lower with MOR20 than with CM commencing 60 minutes after the start of the meal. At 90 and 150 minutes, we saw a smaller increase in blood glucose from baseline with MOR20 as opposed to CM. Compared to CM, MOR20 produced a decreased mean glycemic meal response.

4.0 Discussion:

In this investigation, we present the first proof of a hypoglycemic impact of commercially manufactured MO leaf powder in the Ambal region on the postprandial glucose response in both diabetic and healthy participants when added to a local culinary preparation. We first assessed the nutritional-chemical makeup of the commercially available MO leaf powder and its sensory acceptability in regional meal preparations in order to accomplish this aim.

Additionally, it was discovered that the leaf powder was high in calcium, potassium, and iron, and naturally, this amount was higher than that of the leaves of MO trees grown in other nations [4,5]. The range of the total polyphenol content, which varies greatly depending on the geographic origin, age, wild or domesticated plant status, and method of analysis, was within the range described in the literature for various samples of Moringa leaves. Previous studies also demonstrated that the extract decreased the activity of α-amylase, a crucial enzyme that breaks down dietary carbohydrates into glucose, in the in vitro inhibition test. This suggests that MO leaf powder decreased postprandial glucose levels by slowing down intestinal absorption of glucose and amylase-mediated starch hydrolysis.

Several studies and publications have recommended using MO leaves in conjunction with other regional foods to improve the diets of those in underdeveloped nations by adding more nutrients and bioactive substances. It is crucial to assess the sensory acceptability of a conventional meal enhanced with MO leaf powder, nevertheless, because of its bitter flavor. The primary reason why clinical trials fail is because people don't enjoy the taste of MO leaves in meals. However, there are very few research that have evaluated the sensory acceptability of MO leaves in cuisine. In line with recent research demonstrating a decline in the acceptability of sensory qualities of foods containing Moringa, our test reveals that adding 25 g of MO leaf powder, or 5% of the meal's weight, significantly reduced meal acceptability in terms of color and taste but not texture [7]. However, we discovered that the taste panel approved of the dish that had MO leaf powder added. In a another trial, a group of Ghanaian babies and their caretakers largely approved of the inclusion of 35 g, or 15% of the meal's weight, of MO leaf powder to a meal made of a cereal-legume blend [3]. Consequently, our results are consistent with those found in the literature. However, because of the bitterness of the leaves, it is quite probable that higher concentrations of MO leaf powder provide an overly disagreeable flavor.

Lastly, we assessed how a meal supplemented with MO leaf powder affected the postprandial glycemic response in both healthy and diabetic participants. We discovered that in diabetic participants, supplementing with leaves resulted in a decreased increase in postprandial blood glucose 90–120 minutes after the start of the meal. When compared to the control meal, diabetic patients' mean change in postprandial glucose concentration from the baseline was ≈20–30 mg/dL lower when the meal was supplemented with MO leaf powder. In contrast, non-diabetic patients had no impact.

MO has been proven in previous in vivo experiments to lower postprandial hyperglycemia in mice, either by pre-treating the animals with MO over a specified length of time [11] or by administering a single dose of MO concurrently with the meal [9]. Consuming 50 g of MO boiled leaves together with a typical meal significantly reduced blood glucose levels by 21% one hour after the start of the meal, but the change was no longer significant after two hours, according to a single dosage research conducted on six type 2 diabetes individuals in humans [14]. Furthermore, there was no change in insulin secretion. A lower postprandial glucose response was seen in our study when 20 g of MO leaf powder was added to the meal. This effect persisted for up to three hours after the meal started. Therefore, the amount of leaves given throughout the research and the method of administration may be connected to this distinct effect of MO on glycemic response. In fact, 50 g of fresh leaf is equivalent to 11–12 g of dried leaf, which is less than what was utilized in our study, when a moisture content of 76–78% is taken into account. Additionally, adding the leaf powder to the food after it has been cooked will preserve the nutrients and bioactive components that are lost during cooking.

It has been hypothesized that the presence of fiber and other secondary metabolites in the leaf is responsible for the hypoglycemic action of MO leaf powder. In fact, the high fiber content of MO leaves slows the intestinal absorption of glucose and the time it takes for the stomach to empty. Rather, since they inhibited α-glucosidase and α-amylase, secondary metabolites such as flavonoids and phenolic acids have been implicated in the metabolism of carbohydrates [15,16]. Through hydrophobic bonding, rutin immediately bound to the enzyme and reduced α-glycosidase activity in vitro [7]. Additionally, by attaching to the enzyme through hydrogen bonds and van der Waals forces, kaempferol changed the structure of α-glucosidase and decreased its activity in vitro [8]. Through the suppression of both maltase and sucrase activities in vitro and in vivo, quercetin demonstrated greater inhibitory actions [9]. Furthermore, when quercetin was given orally with glucose as opposed to glucose alone, it decreased postprandial blood glucose levels in diabetic mice and blocked GLUT2-mediated absorption in vitro. Similarly, by inhibiting the sodium-dependent SGLT1-mediated glucose transporter, some phenolic acids, such as chlorogenic, ferulic, caffeic, and tannic acids, may likewise affect the intestinal absorption of glucose [1]. One potential reason for the postprandial glucose response results is the suppression of amylolytic enzymes, which slows down the digestion of carbs and lowers the pace at which blood glucose increases. Other secondary metabolites found in MO leaves are called isothiocyanates. These substances, which are produced by the hydrolysis of glucosinolates by myrosinase, have been shown to have anti-diabetic effects by inhibiting the gluconeogenesis of the liver [2,3].

It is conceivable that consistent usage of MO leaf powder might enhance the control of glycaemia in diabetic people since it can lower postprandial glycemic response. Little research has been done on how consuming MO leaf powder on a regular basis affects blood glucose levels in people. If proven, this kind of action would be exclusive to groups that have less access to Western medication treatments for political, economic, and environmental reasons. The diabetic community, for example, is a traditionally nomadic group that has not seen economic growth and relies on food aid to survive. For this demographic, a typical food aid basket consists of sugar and starchy foods (refined grain cereals, legumes, and blended meals), with little to no fresh or dry fruit and vegetables. This imbalanced low-diversity diet is likely the primary source of the high prevalence of stunting and general and central obesity seen in women and diabetes patients, respectively [2], as well as the nutritional inadequacies, particularly of vitamins and minerals, seen in youngsters [4]. Type 2 diabetes is mostly brought on by obesity and a high intake of processed sugars and carbs. Diabetes is a common health issue among the populace, and it is often managed with diet or diet plus hypoglycemic medications. In this situation, MO leaves could be utilized as a dietary supplement to help diabetic patients regulate their blood sugar levels and enhance the quality of their meals. In order to assess the sensory acceptability and blood glucose effects of a meal supplemented with locally produced MO leaf powder, we chose diabetics as the target population. This stands for our study's first strength. Second, we assessed the hypoglycemic effects of MO leaf powder in humans on a constant number of patients with and without diabetes for the first time. Our study does have several limitations, though. For starters, we tested blood glucose in capillaries rather than venous veins. Capillary blood glucose measurement is a quick and easy method that has been chosen for its special convenience because of the low availability of laboratory equipment and reagents on site, even though comparison studies have revealed slight but significant differences in the glycemic values obtained from the two methods [5,6]. The second drawback of our study is the inability to quantify the insulin response to the meal for the same reason. Lastly, we neglected to document the individuals' actual food intake the night before the test. But since the food provided in the genrally home made is the same as that seen in food assistance packages, we may presume that the participants ate about the same things.

Author Contributions

Conceptualization, Formal analysis, Methodology, P.K Writing—original draft,.

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Conflicts of Interest

The authors declare no conflict of interest.

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