Ann Res Antioxid. 2020;5:e02 Original

Annals of Research in Antioxidants http annresantioxidants.com

Evaluation of hypoglycemic and hepatoprotective activity of *Moringa oleifera* and *Morinda citrifolia* leaf extracts in diabetic rats; a comparative study

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Received 21 Aug. 2020
Accepted 20 Sep. 2020
ePublished 6 Oct. 2020

Keywords: Type 2 diabetes, *Moringa oleifera,* Morinda citrifolia

Abstract

Introduction: *Moringa oleifera* and *Morinda citrifolia* are the medicinal plants used to treat diabetes and liver diseases.
Objectives: This study was done to evaluate and compare the hypoglycemic and hepatoprotective activity of *Moringa oleifera* and *Morinda citrifolia* leaf extracts using male Sprague Dawley rats.
Materials and Methods: Healthy Sprague-Dawley male rats weighing 200 to 220 g were used for the study. Animals were randomly distributed into five groups of five for each; control (C), diabetic control (DC), diabetic standard (DS), *Moringa oleifera* (MO) and *Morinda citrifolia* (MC). Diabetes was induced using 50 mgs of streptozotocin/kg body weight. After two days of streptozotocin induction, animals with blood glucose value of 190 mg/dL and above were considered diabetic. The animals of MO and MC treated groups were fed with 300 mgs of *M. oleifera* and *M. citrifolia* leaf extract respectively, along with normal food and water *ad libitum*. Meanwhile the animals of DS group were administered with glibenclamide at a dose of 2.5 mg/kg/d with food while animals of group DC and C were left untreated. Blood glucose levels and body weight of all the animals were measured at Days 1, 7 and 14.
Results: Diabetic experimental animals treated with *M. oleifera,* showed a significant decrease in fasting glucose from 236 mg/dL (day 1) to 171 mg/dL (day 14), and this represented a decrease of 26 %. The animals treated with *M. citrifolia* showed a decrease in fasting blood glucose from 257 to 169 (34 %). The diabetic standard group showed the reduction in fasting blood sugar from 251mg/dL to 134 mg/dL (47%). The liver enzymes remained normal in the extract treated animals and the histology report of the liver tissue did not show any abnormal features.
Conclusion: The data of this study showed that *M. citrifolia* has higher hypoglycaemic activity than *M. oleifera* without having toxic effect on liver. Therefore, our study recommends the use of both extracts either individually or in the ratio of 1:1 mixture to manage the diabetes.

Citation: Nayak BS, Rodrigues V, Rao AV C. Evaluation of hypoglycemic and hepatoprotective activity of *Moringa oleifera* and *Morinda citrifolia* leaf extracts in diabetic rats; a comparative study. Ann Res Antioxid. 2020;5:e02.

Introduction

Type 2 diabetes mellitus (T2DM) can be considered an epidemic crisis within the North America and Caribbean regions of the world. Diabetes mellitus (DM) is an execrable, heterogeneous non-communicable disease, which has attained an epidemic proportion worldwide (1,2). As of 2017, the prevalence of diabetes was 8.8 %, which is expected to increase to 9.9 % by 2045 (3). DM is a group of common metabolic disorders in which blood glucose concentration is persistently above the normal ranges (4,5). The major implications for onset of T2DM include lifestyle, senescence and genetic disposition of the individual. Obesity, sedentary lifestyles, smoking, unbalanced diets or overconsumption of alcohol are all factors contributing to this disease (6). Several genes such as WFS1, which regulates calcium or FTO associated with obesity along with thirty-eight genetic variants, highly inheritable, influence the onset of T2DM. Focusing on the pathophysiology of T2DM highlights insulin resistance, resulting in reduction of insulin production and failure of the beta cells of the pancreas. Hence, glucose is no longer assimilated efficiently resulting in a hyperglycemia (7). Pharmaceuticals such as metformin, function at the liver, improving peripheral

Core tip
M. citrifolia has higher hypoglycaemic activity than *M. oleifera* without having toxic effect on liver

insulin sensitivity, without inducing weight gain or risk of hypoglycemia while ensuring cardiovascular safety. The main component of metformin stems from the *Galega officinalis*. The components of this plant include guanidine which synthesizes a class of biguanide, thus, ensuring antidiabetic properties (8).

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for hyperglycaemia. There is a growing interest in herbal remedies because of their effectiveness and without any complications in clinical experience. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown (5). Therefore, studies with plant extracts are useful to know their role, mechanism of action and safety.

Moringa oleifera is a fast-growing, drought-resistant tree of the family Moringaceae, native to the Indian subcontinent. Common names include moringa, drumstick tree**.** *M. oleifera* leaf extract has been tested to provide a purgative effect, relief for headaches, antimicrobial activity for sores, bronchitis, eye and ear infections and scurvy. Furthermore, due to the presence of thiocarbamate glycosides, the extract has been seen to reduce high blood pressure levels. Meanwhile, the availability of β-sitosterols functions as a cholesterol reducing agent. The presence of antitumor properties due to niazimicin found in the leaf extract which functions as a chemo preventive agent (9).

Morinda citrifolia L (Rubiaceae) also known as noni, or Indian mulberry, is a small evergreen tree. It is native to the Pacific islands, Polynesia, Asia and Australia. The leaves are 8 or more inches long and are oval in shape. *M. citrifolia* has been observed and tested to contain the same antimicrobial, antitumor, antioxidant properties as *M. oleifera.* Containing similar β-sitosterols compounds along with anticancer properties and additives of chlorophyll aiding in glucose level reduction (10)

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Objectives

There are studies which reported on the hypoglycaemic activity of M. *oleifera* and *M. citrifolia* and very little data available on the comparison of these two extracts which people use for managing their diabetes along with the prescribed hypoglycaemic agents. Therefore, this study is designed to study the hypoglycaemic activity of both the leaf extracts to know which one is more effective in treating diabetes.

Materials and Methods

Extract preparation

The *M. oleifera* and *M, citrifolia* leaves were obtained from Isitor Agromed Technology Ltd. This was grounded into a powder weighing 5 grams each and macerated in 70% ethanol overnight. Filtration was carried out utilizing Whatmann no.1 branded filter paper and left under the fume hood overnight. The dark, moss green concentrated extracts obtained was stored in a container at 8-10°C refrigerator.

Phytochemical screening methods

Saponins

Extract (300 mg) was boiled with 5 ml water for two minutes; the mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicates the presence of saponins (11).

Tannins

To 1mL of extract (300 mg/mL) was added to 2 mL of sodium chloride (2%), filtered and mixed with 5 mL 1% gelatin solution. Precipitation indicates the presence of tannins (11).

Triterpenes

Extract (300 mg) was mixed with 5 mL chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes (12,13).

Alkaloids

Extract (300 mg) was digested with 2 M HCl, and the acidic filtrate was mixed with amyl alcohol at room temperature. Pink colour of the alcoholic layer indicates the presence of alkaloids (13, 14).

Flavonoids

The presence of flavonoids was determined by using 1% aluminum chloride solution in methanol, concentrated HCl, magnesium, and potassium hydroxide solution (11).

The thin layer chromatography of the aqueous extract on silica gel was done using the medium Chloroform: methanol (9:1 vol/vol) and Chloroform: acetone (1:1 vol/vol) as the mobile phase.

Animals

Healthy adult Sprague-Dawley male rats weighing between 200 and 220g were used for the study. The animals were individually housed and maintained on normal food and water *ad libitum*. Animals were periodically weighed before and after the experiments. Fasting blood sample was collected from tail vein of the rats. The glucose levels of these samples were determined using the glucometer, which employed the glucose oxidase/peroxidase reaction. All animals were closely observed for any infection, and those that showed signs of infection were separated and excluded from the study.

Diabetes was induced by administering 50 mg/kg of Streptozotocin (15) in cold citrate buffer, pH 4.5, intraperitoneally to overnight fasted rats. After two days, animals with a fasting blood glucose >190 mg were considered diabetic. Then the rats were grouped into five groups of 5 each: The first group (control) and second

group (diabetic control) received food and water *ad libitum*, throughout the duration of the study, third group, diabetic standard (DS) which received glibenclamide at a dose of 2.5 mg/kg/d, fourth and fifth group received *M. oleifera* (MO) and *M. citrifolia* (MC) extract respectively as a dose of 300 mg along with food and water *ad libitum* (extract was administered by coating the granules of chow). Blood glucose levels and weight of all the animals were measured at day 1 (baseline), 7 and 14.

The rats were euthanized on the day 14 by enclosing in a chloroform chamber. Once unconscious, they were injected through the heart to obtain the final blood sample. Blood samples were analysed for serum aspartate aminotransferase (AST) and alanine aminotransferase (AST). The liver samples were taken after euthanisation for the histological evaluation.

Ethical issues

This experimental study was approved by the Ethics Committee of Faculty of Medical Sciences Medical Sciences, The University of the West Indies (# EC-A/5-2019) and was in accordance with the international guidelines for the care and use of laboratory animals.

Statistical analysis

The mean plus or minus was determined analysed using STAT 14.2. Repeated measure of two-way ANOVA was used to compare the groups (SPSS version 16.0, Chicago, USA). Differences between groups were considered significant at $P < 0.05$ levels.

Results

The preliminary study conducted with *M. oleifera* showed the presence of alkaloids, triterpenoids, saponins and flavonoids. The *M. citrifolia* showed the presence of alkaloids, tannins, flavonoids and triterpenoids.

Fasting blood glucose

There was a significant decrease in fasting blood sugar, observed in diabetic experimental animals treated with *M. oleifera* and *M. citrifolia* and the diabetic standard with reference to hypoglycaemic drug, glibenclamide as compared to diabetic untreated animals. In diabetic experimental animals treated with *M. oleifera*, there was a significant decrease in fasting glucose from an excess of 236 mg/dL (236.8 ± 62.84) (day 1) to 171 mg/dL (171.8 ± 52.69) (day 14), and this represented a decrease of 26 %. The animals treated with *M. citrifolia* showed a decrease in fasting blood glucose from 257 (257.4 ± 64.54) (day 1) to 169 (169.6 ± 47.76 (day 14), and this represented 34 %. The diabetic standard group showed the reduction in fasting blood sugar from 251 mg/dL to 134 mg/dL (251.8 ± 65.18 – day 1, 134 ± 16.67 – day 14) and this represented 47% (Table 1). The decrease in fasting blood glucose levels observed with the untreated diabetic animals was not significant (Figure 1).

Body Mass

In the latter 14 days of the treatment period, there was not much variation in the body weight in the diabetic experimental and diabetic standard group animals when compared to control and diabetic control (Table 2 and Figure 2).

Liver enzymes

Liver enzyme (AST and ALT) levels were within the range with the blood samples obtained from all the five groups of animals. This supported the non-toxic effect of *M. oleifera* and *M. citrifolia* extracts on liver (Figure 3).

Histological analysis

Histological analysis of the liver tissue obtained from the extract treated animals showed a steady glycogen content and with mild to no inflammation. This indicated that *M. oleifera* and *M. citrifolia* are not having any liver damaging activities (Figure 4).

Discussion

This study demonstrated the hypoglycaemic activity of *M. oleifera* and *M. citrifolia* without having any adverse effects on liver function. The phytochemical constituents of *M. oleifera* and *M. citrifolia* might have lowered

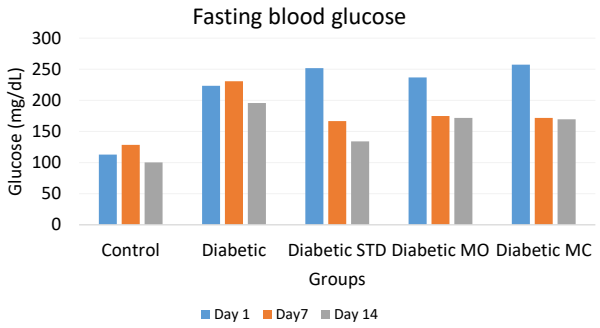

Figure 1. Fasting blood glucose values of five groups of animals on day 1, 7 and 14.

Table 1. Fasting blood glucose values of five groups of animals on day 1, 7 and 14

Days	Control	Diabetic	Diabetic Standard	Diabetic MO	Diabetic MC
Day 1	112.8 ±25.38	223.4±39.68	251.8±65.18	236.8±62.84	257.4±64.54
Day7	128.4±97.2	230.8±113.35	166.6±65.34**	174.8±81.1*	171.8±52.68*
Day 14	100.2±17.50	195.6±53.75	134±16.67**	171.8±52.69*	169.6±47.76*

Values are mean ± SD, n=5 * $P < 0.05$, ** $P < 0.01$.

Table 2. Body weight of five groups of animals on day 1, 7 and 14

Days	Control	Diabetic	Diabetic Standard	Diabetic MO	Diabetic MC
Day 1	295.6 ±45.14	189.8±13.5	187.8±21.5	200±8.5	188.8±17.9
Day7	210.6±100.2	214.4±33.4	200.8±46	223.8±40.5	229.4±19.1
Day 14	240.4±26	237.4±34.32	224.6±56.0	235.0±46.3	252.2±15.3

Values are mean ± SD, n=5.

glucose levels either by promoting insulin secretion or by increasing insulin receptor sensitivity like biguanides and glitazones. Recently, scientists showed a similar phenomenon of hypoglycaemic action with the extracts of *Cynodon dactylon* (16). Other researchers also showed higher level of hypoglycemic activity of *M. oleifera* and *M. citrifolia* with a dose of 500 mg and longer duration of treatment (70 days) when compared to the dose of 300 mg and 14 days of treatment which we performed in our experiment. (17,18). The length of treatment might have played the role in higher level of hypoglycaemic activity of the extracts.

Diabetic standard group, treated with glibenclamide exhibited a significant reduction in the glucose values. Thus indicating the efficiency of the drug in the control of T2DM (19). Animals treated with extracts and standard drug showed normal concentrations of glycogen. This highlighted the effectiveness of the drug in promoting the action of insulin to store the glucose in the form glycogen in the liver. This supported non-toxic effect of *M. oleifera* and *M. citrifolia* on liver cells. Wang et al demonstrated the hepatoprotective activity of noni fruit juice against CCL4 induced liver damage (20).

The control group animals maintained their blood sugars below 130 mg/dL because of the normal functioning of insulin in up-taking of excess carbohydrates to be stored as glycogen. The diabetic control group animals showed increased level of blood glucose even after 14 days without any treatment. These findings were consistent with the similar research carried out under the same conditions (21) .

The hypoglycaemic activity, exhibited by the *M. oleifera* and *M. citrifolia* leaf extracts, may be attributed to the presence of triterpenes and saponins. Other researchers also demonstrated the presence of a significant quantity of bioactive compounds like flavonoids, triterpenoids, triterpenes, and saponins in *M. citrifolia* (22,23). It has been suggested that saponins may significantly inhibit gastric emptying (24,25). The saponins could inhibit gastric emptying either by promoting secretion of glucagon like peptides-1 or by inhibiting its degradation. This drug-induced gastroparesis is an effective method of managing hyperglycaemia because it slows the process of nutrient absorption into the blood stream. Also, the presence of

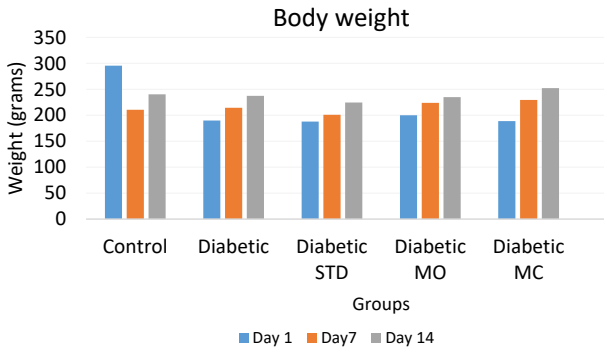

Figure 2. Body weight of five groups of animals on day 1, 7 and 14.

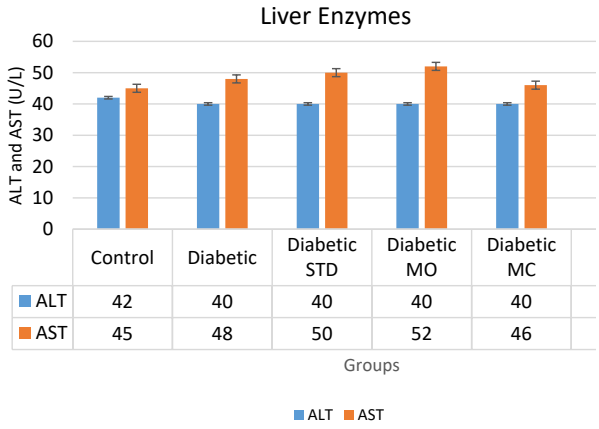

Figure 3. Liver enzymes of five groups of animals.

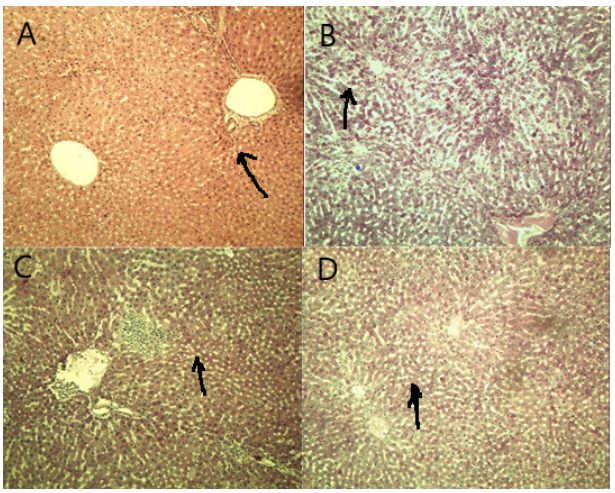

Figure 4. A: Histology of the liver specimen obtained from normal control animal (PAS-stain). **B:** Histology of the liver specimen obtained from diabetic standard animal. **C:** Histology of the liver specimen obtained from M. oleifera treated animals. **D:** Histology of the liver specimen obtained from M. citrifolia treated animal. All are showing mild to no inflammation and well distributed glycogen granules throughout the slide area.

saponin in *M. citrifolia* may have a glucagon decreasing effect and may enhance glucose utilization lowering blood glucose. It is also reported that saponin stimulates insulin release from the pancreas (26), and it could be due to decreased degradation of glucagon like peptides. On the other hand, glibenclamide exerts hypoglycaemic action by stimulating insulin secretion and inhibiting glucagon release. The remaining intact pancreatic cells are stimulated by *M. citrifolia* or glibenclamide, and the serum insulin level is increased, and the blood glucose is decreased. Rutin is a flavonoid (a glycoside composed of rutinose and quercetin) found in significant quantities in the *M. citrifolia* fruit, and it is postulated that the rutinose residues may act as a secretagogue, which potentiates insulin secretion by a mechanism related to that of sugar sucrose. Triterpenoids have also been indicated as possible therapeutic agents that can be beneficial in the management of diabetes mellitus, as they have been shown to be effective in improving symptoms of glycosuria and blood sugar in alloxan-induced mice (27,28).

Conclusion

The data of this study showed that *M, citrifolia* has higher hypoglycaemic activity than *M. oleifera* without having toxic effect on liver. Therefore, our study recommends the use of both extracts either individually or in the ratio of 1:1 mixture to manage the diabetes.

Acknowledgements

The authors would like to express their sincere thanks to the Campus Research and Publication Fund, The University of the West Indies, St Augustine, Trinidad for providing the grant to conduct this study. Authors also thank animal house of the Veterinary Sciences for care and housing of the rats under study and the biochemistry unit, faculty of medical sciences for their facilities and resources. Authors also would like to thank Ms Sabiha Mohammed and Mr Shankar Madivala, Department of Preclinical sciences for their help during the study.

Authors' contribution

All the authors contributed to data collection and preparation of the manuscript. The first draft was prepared by NB. All authors read the final version and confirmed for the publication.

Conflicts of interest

Authors declare there is not any conflict of interest.

Ethical considerations

Ethical issues including text plagiarism, misconduct, manipulation or appropriation, data fabrication, falsification, redundant publication as well as duplicate submissions have been carefully observed by authors.

Funding/Support

This study was supported by The Campus Research and Publication Fund, The University of the West Indies None, St. Augustine, Trinidad.

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