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Consumption of *Moringa oleifera* flour and its effects on the biochemical profile and intestinal motility in an animal model

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Abstract

Moringa oleifera (MO) belongs to the family *Moringaceae* and is native to tropical Africa. It exhibits many therapeutic properties and has been widely cultivated because of the high food value of the leaves, fruit, flowers and roasted seeds. It possesses high quality protein, calcium, iron, fiber, minerals and essential amino acids. The objective of this study was to evaluate the effects of MO flour in anthropometric and biochemical profile of Wistar rats. Animals were divided randomly into the following groups (n=10): G1 (control group) and G2 (treated with MO flour mixed to the rat food) were both treated for 40 days and G3 (control group), G4 (treated with senne) and G5 (treated with MO flour) were studied to evaluate the intestinal motility. After 40 days, animals of G1 and G2 were euthanized and evaluation of glycaemia, total cholesterol, triglycerides, LDL-c, VLDL-c, HDL-c, C reactive Protein (PCR), hepatic enzymes, Lee Index, weight and visceral fat were performed. Our results showed reduction of visceral fat, total cholesterol, triglycerides, LDL-c, and VLDL-c and increase in the HDL-c levels. No significant differences were found in the body weight, glycaemia, hepatic enzymes and PCR. The MO flour also promoted laxative effects similar do senne. Our results with the use of *Moringa oleifera* flour are very promising, once its use improved lipid profile, prevented weight gain and showed no adverse effects. Thus we may conclude that this flour could be added to industrial products in order to provide healthier products to the consumers.

Keywords: *Moringa oleifera*, Wistar rats, glycaemia, cholesterol, visceral fat, intestinal motility

Introduction

Plant-based therapies have become increasingly popular for the prevention or as an adjuvant to treatment of many diseases. Besides, the lower costs, when comparing to traditional allopathic medicines, allow them to be an interesting alternative to low-income communities. Thus, many authors have dedicated to study the actual effect of medicinal plants in promoting health or preventing risk factors for chronic diseases, such as diabetes, metabolic syndrome and cardiovascular diseases,that are the main causes of death nowadays. [1-3]

Moringa oleifera (MO) Lam, belongs to the family Moringaceae and is native to tropical Africa. It shows rapid growth, reaching up to 10 meters high and is it is mostly cultivated in tropical and subtropical areas as a valued plant. As well as having various therapeutic properties, it is also cultivated because of its high nutritious value of the leaves, fruit, flowers and roasted seeds, since they have high quality calcium, iron, protein, fiber, minerals and essential amino acids. The leaves are good source of folic acid, riboflavin, nicotinic acid, pyridoxine, vitamins A, B, C and E, proteins, amino acids and phenolic compounds and minerals. MO leaves are highly nutritious, being a significant source of sterols, alkaloids, glycosides, phenolic and flavonoids. [4-7]

MO is used in popular medicine to treat inflammation and infectious processes, hepatic, gastrointestinal, cardiovascular and hematological disorders on account of its anti-oxidant, anti-inflammatory, anti-hypertensive, anti-tumor, cholesterol and glycaemia lowering, and hepatoprotective effects. [5-12] It can be widely used by the pharmaceutical and food products.

Due to the close association of lifestyle and the occurrence of chronic diseases, many industries have been trying to produce foods nutritionally enriched. The flour produced from medicinal plants can be employed in the preparation of pasta, breads, cakes, snacks, and several other products. In accordance with this tendency studies need to be performed in order to satisfy the industry needs, thus, the aim of this research was to verify the effect of *Moringa oleifera* flour on the metabolic profile and intestinal motility of Wistar rats.

Materials and Methods

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Preparation of the *Moringa oleifera* flour

Moringa oleifera leaves were removed from the branches and rinsed in distilled water and immersed in a solution of 200 mL of sodium hypochlorite distilled water (1:1) and later dehydration in a ventilated oven at a temperature of 60 C for 24 hours. Then the material was crushed in the mill (Model MA090 - Marconi ®), screened at 60 mesh sieve, and the flour obtained was wrapped in glasses with airtight lids

Preparation of the supplemented rat feed

The rat feed was prepared weekly, in a proportion of 30% and70% flour/commercial rat feed.
The commercial rat feed was crushed in a plastic mortar and pestle, and mixed with the *Moringa oleifera*. After weighing the components separately on a semi-analytical balance, they were mixed homogeneously, using water as binder. Using a meat-filling machine, this mixture was molded into a cylindrical shape identical to that of the commercial feed. The resulting pellets were dried in an air circulating oven at 50 C for about 48 hours, stored in polyethylene packaging, and refrigerated at 5 C until its utilization.

Evaluation of the percent composition of the MO flour, rat feed and rat feed added with the flour

The MO flour, rat feed and rat feed added with the flour were studied (in triplicate) in terms of its moisture content (total dry extract) by gravimetric method in an oven at 105 C for 16 h until it reached a constant weight. Concentration of lipids was evaluated by Soxhlet extraction. Total nitrogen was evaluated by the Kjeldahl method (multiplying the values of total nitrogen by 6.25 to obtain the equivalent values in protein). Ashes were analyzed in a muffle furnace at 550 C. Carbohydrates were evaluated by difference, as well as crude fiber. [13]

Animal groups

This research was approved by the Animal Research Ethics Committee of the University of Marília (UNIMAR/ Marília, SP, Brazil). Male Wistar rats were used, weighing approximately 230g to 250g, which were kept in the vivarium at UNIMAR. The rats were housed in collective cages under a dark/light cycle of 12 hours, room temperature of 22 ± 2 C, and relative air humidity of 60 ± 5%. Throughout the experiment, the animals were fed and watered *ad libitum*; *and* were cared for according to the recommendations of the Canadian Council's "Guide for the care and use of experimental animals".
After seven days of acclimation to the laboratory conditions, the rats were divided randomly in two experimental groups (n=10 per group): as following

G 1: Control group that was fed water and rat food *ad libitum*;
G 2: Treated group that was fed water and rat food supplemented with *Moringa oleifera ad libitum*.
The weight gain was evaluated once a week and the consumption of the animals was recorded based on the leftovers found each day.

Blood collection and evaluation of the biochemical profile, Atherogenic index, and Protection Index

After 40 days of treatment and a 10-hour fast, the animals of G1 and G2 were euthanized with a lethal intraperitoneal injection of thiopental (200 mg/Kg) until complete sedation. After that blood samples were drawn from the vena cava to determine the biochemical profile: total cholesterol, High Density Lipoprotein (HDL-c), Low Density Lipoprotein (LDL-c), Very Low Density Lipoprotein (VLDL), triglycerides, glycaemia, high sensitivity Protein C Reactive (hsPCR); aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The glucose, lipid levels and hsPCR were measured in mg/dL; AST and ALT in U/L. Atherogenic Index (AI) and Protection Index (PI) were performed after Schulpis, Karikas [14] and Munshi, Joshi, Rane [15]: AI = (Total cholesterol – HDL-c)/HDL-c. PI is calculated by using: {[AI (G1)- AI (G2)]/AI (G1)}x100.

Determination of body composition parameters and evaluation of visceral fat

At the end of the experiment, all the animals were anesthetized prior to measuring their body length (nose-to-anus or nose–anus length). The body weight and length were used to determine the Lee index which is the cube root of body weight (g) / nose-anus length (cm), and weight gain (in percentage). When values are above 0.3, it is possible to postulate overweight in the animals. [16,17]
We also made an incision in the abdominal region and the visceral fat was removed with scissors and tweezers and weighed.

Intestinal motility test

The intestinal motility test was evaluated according to the model described by Michelin, Salgado [18], with modifications. *Cassia angustifolia* suspension was used as comparison for the results. After a 24-hour fast, three groups (n=10 per group) were gavage-fed, according to:
G3: Control group, treated with 0.2mL of propylene glycol;
G4: Group treated with 0.2mL *Cassia angustifolia* (senne) suspension prepared with propylene glycol, 50mg/mL (dose of 30mg/kg);
G5: Group treated with 0.2mL of *Moringa oleifera* flour suspension prepared with propylene glycol, 50mg/mL (dose of 30mg/kg).

Forty five minutes later, the groups were gavage-fed 0.2mL of a 10% activated charcoal suspension in 5% gum arabic. Two hours after the administration of activated charcoal, the animals were euthanized with a lethal intraperitoneal injection of thiopental (200 mg/Kg). After death was confirmed, the intestines were removed and their length and the distance traveled by the activated charcoal of each group were measured.

Statistical analysis

Kruskal-Wallis supplemented with Dunn test were used for the statistical analysis and the variables are presented as mean and standard error mean, adopting a 5% level of significance.

Results

Our results did not show differences among food intake (table 1) and percentage of weight gain in the animals from the control group (G1) and those treated with *Moringa* (G2) (table 2). Nevertheless, the visceral fat weight is higher in the control group (G1) compared to G2 (table 3). Lee Index was higher in the control group when compared to the group treated with MO.

Table 1 – Mean and standard deviation of food intake by the Animals of the Control Group (G1) and *Moringa oleifera* group (G2) during the treatment.

G1	G2
345.84 ± 143.58 (A)	314.78 ± 136.79 (A)*

(*)Same letters indicate a significant difference between the treatments at a level of 5%.

Table 5 shows the results for the biochemical parameters. *Moringa oleifera* flour decreased levels of total cholesterol, triglycerides, LDL-c, and VLDL-c and increased levels of HDL-c. No significant differences were found in the glycaemia, hsCRP, AST and ALT. Table 6 shows that AI is higher in the control group. Results for the percentage of cardiovascular protection (PI), shows that MO the value of 99,84%.

Table 2 – Mean and standard deviation of the percentage of weight gain in the Control Group (G1) and in the group treated with *Moringa oleifera* (G2).

G1	G2
55.37 ± 6.51 (A)*	53.75 ± 4.10 (A)

(*)Same letters indicate no significant difference between the treatments at a level of 5%.

Table 3 – Mean and standard deviation of the weight of the visceral fat in the Control Group (GC) and in the group treated with *Moringa oleifera*.

G1	G2
5.48 ± 2.30 (B)*	2.97 ± 0.64 (A)

(*)Different letters indicate a significant difference between the treatments at a level of 5%.

Table 4 – Mean and standard deviation of the Lee Index in the Control Group (GC) and in the group treated with *Moringa oleifera*.

G1	G2
0.276 ± 0.01 (B)	0.228 ± 0.02 (A)*

(*)Different letters indicate a significant difference between the treatments at a level of 5%.

Table 5 – Mean and standard deviation of the biochemical parameters the Animals of the Control Group (G1) and *Moringa oleifera* group (G2).

Parameters	G1	G2
Glycaemia (mg/dL)	138.73 ± 55.79 (A*)	159.30 ± 29.43 (A)
Total cholesterol (mg/dL)	53.55 ± 4.95 (B)	48.00 ± 3.9 (A)
Triglycerides (mg/dL)	123.36 ± 23.04 (B)	46.90 ± 7.43 (A)
HDL-c (mg/dL)	32.64 ± 2.62 (A)	48.80 ± 6.07 (B)
LDL-c (mg/dL)	28.00 ± 8.16 (B)	12.90 ± 4,41 (A)
VLDL-c (mg/dL)	16.73 ± 8.08 (B)	10.80 ± 4.49 (A)
hsPCR (mg/dL)	0.07 ± 0.04 (A)	0.10 ± 0.01 (A)
AST (U/L)	117.27 ± 29.08 (A)	108.10 ± 43.33 (A)
ALT (U/L)	70.45 ± 15.41 (A)	60.0 28.33 (A)

(*)Different letters indicate a significant difference between the treatments at a level of 5%. LDL-c: Low Density Lipoprotein; VLDL-c: Very Low Density Lipoprotein; HDL-c: High Density Lipoprotein; hsPCR: high sensitivity Protein C Reactive; AST: aspartate aminotransferase and alanine ALT: aminotransferase.

Table 6 - Atherogenic Index (AI) in groups G1 (control group) and G2 (*Moringa oleifera*)

	G1	G2
AI	0.196	0.001

Table 7 presents the percentage of the distance covered by the activated charcoal from the pylorus to the beginning of the caecum. Significant differences were found in the control group (G3), group treated with *Cassia angustifolia* (senne) (G4) and the group treated with *Moringa*. Higher values were found in the animals treated with *Moringa*.

Table 7 – Mean and standard deviation of the distance covered by the activated charcoal from the pylorus to the beginning of the caecum (G3: group treated with propylene glycol; G4: group treated with senne and G5: group treated with *Moringa oleifera*).

G3	G4	G5
73.79 ± 8.13 (A)	84.54 ± 5.55 (B)	90.97 ± 2.30 (C)*

(*)Different letters indicate a significant difference between the treatments at a level of 5%.

After the period of treatment, we observed hyperpigmentation of the gut in the group treated with MO. These alterations were not observed in the control group.

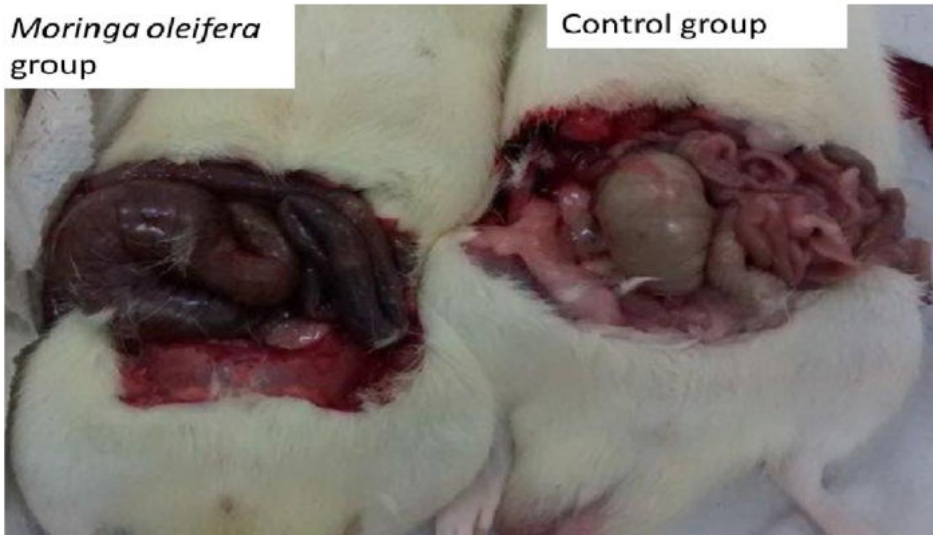

Figure 1: Morphological aspect of the gut from animals of control group (G1) and Group treated with *Moringa oleifera* flour.

Table 8 shows the composition of the MO flour and commercial rat feed mixed with the flour.

Table 8 – Means and standard deviation of the composition of *Moringa oleifera* flour, commercial rat feed and commercial rat feed (70%) mixed with of *Moringa oleifera* flour (30%).

Samples	Moringa	Rat feed	Rat feed 70% + Moringa flour 30%
Energy value (kcal)	303.63 ± 1.93	350.06 ± 1.15	343.72 ± 0.15
Moisture at 105 C (%m/m)	5.96 ± 0.19	9.60 ± 0.09	10.36 ± 0.08
Ashes (%m/m)	9.31 ± 0.11	7.32 ± 0.14	8.02 ± 0.05
Carbohydrates (%m/m)	41.66 ± 1.32	57.15 ± 1.09	56.62 ± 1.01
Proteins (%m/m)	24.14 ± 0.96	22.38 ± 0.88	21.55 ± 0.93
Total Fat (%m/m)	4.49 ± 0.25	3.55 ± 0.29	3.45 ± 0.07
Crude Fiber (%m/m)	14.44 ± 0.27	7.04 ± 0.08	9.15 ± 0.09

Discussion

Animals treated with MO did not exhibit modifications in food intake and in the weight gain percentage when compared to the control (even observing in table 7 that the rat feed mixed with the MO flour has an energy value higher when compared to the regular rat feed).

However, it was observed decrease in the amount of visceral fat and Lee Index. These effects may be interesting because obesity is considered and important cause of type 2 diabetes and cardiovascular disease. Associated with this, the visceral fat is related to deleterious effects on the metabolic and hemodynamic profile and it is closely associated to insulin resistance, diabetes, hypertension, and dyslipidemias and can reduce myocardial perfusion by impairing vascular endothelial function. [19-21] Olayaki et al [22] found similar effects on the anthropometric profile of animals treated with methanolic extract of MO. It is also important to say that this plant is related to a high index of heart protection according to the results of AI (table 6) and PI.

Our results showed improvement in the Lee Index in animals treated with MO. Lee index is an anthropometric evaluation and it is performed in order to identify overweight and obesity in rats [16, 17, 23]. MO flour also improved lipid profile but did not interfere on the glycaemia. Similar to our results Olayaki et al [22] also obtained improvement in the lipid profile and differently from our results, obtained improvement in the glycaemia. Abd et al [24] studied the effects of aqueous extract of MO leaves in diabetes rats and observed benefic effects on the glycaemia. These authors also observed hypolipidemic effects and regeneration of damaged hepatocytes and pancreatic β cells due to antioxidant properties. Kushwaha, Chawla, Kochhar [25] used MO powder in glycaemia and blood levels of antioxidant and markers of oxidative stress and found reduction of glucose levels and antioxidant properties. Chumark et al [26] also studied the effects of MO leaves and their results showed that this plant exhibits antioxidant, hypolipidaemic and antiatherosclerotic properties and authors concluded that it has therapeutic potential. MO leaves are source of sterols, alkaloids, glycosides, phenolic and flavonoids and vitamins C, E, A, caffeoylquinic acids, carotenoids-lutein, alpha-carotene and beta carotene, kaempferol, quercetin, tannins, rutin, and saponins [27-29]. These components may be related to the effects on the lipid profile of the animals treated with the flour. Furthermore, Leone et al [30] found that the leaves are rich in minerals, protein, and β-carotene. As main phenolic compounds authors found quercetin and kaempferol glycosides. They also found salicylic and ferulic acids that are two phenolic acids with pharmacological activity, and therefore they could work as nutraceutical and functional ingredients. Vongsak, Mangmool, Gritsanapan[31] also studied MO leaves and concluded that this plant is good sources of natural antioxidant as isoquercetin. In a very complete review, Stohs, Hartman [32], pointed that almost every part of the MO plant may be used as food products in human nutrition and say that many controlled studies indicate no side effects, thus represents a high degree of safety. Its powder may be used to control glycaemia and dyslipidemia both in human and animal models possibly due to a wide range of biological activities as antioxidant, analgesic, antihypertensive, tissue protective (liver, kidneys, and heart), antiulcer, and others. Authors postulate that these biological functions are related to the presence of several polyphenols and phenolic acids as well as flavonoids, glucosinolates, and alkaloids.

The levels of hsCRP did not modify after using MO flour (table 5). The production of this protein is related to active infection or acute inflammation and therefore it is considered an inflammatory marker usually regulated by Interleucin-1 (IL-1), IL-6 and Tumor Necrosis Factor (TNF-). As it can be useful to detect low grade inflammation condition, the increase of this protein is associated to chronic inflammatory process [33-34]. Despite we have observed inflammatory areas in the gut of G2 (Figure 1), levels of hsCRP did not vary in the groups. The augment of hsPCR levels may occur in patients with major complications in the colon or rectum [35].

The evaluation of hepatic enzymes (AST and ALT) did not show modifications after use of MO flour. When it is observed elevation of these enzymes, destruction of liver cells may be occurring [36], however, our results did not show significant modifications in the group treated with MO flour, suggesting that this flour is safe for consumption. We also performed the acute experiment of intestinal motility of the animals and our results showed that MO may exhibit potential to help intestinal obstipation condition. Our diary observations during the experimental period showed that animals treated with this plant exhibited intestinal motility higher than the control and the results for the acute experiment of intestinal motility showed that MO is more effective than control group and group treated with senne in the intestinal motility. Chronic constipation is related to chronic disease processes, medication use, and psychosocial issues and is responsible for about 8 million annual visits to health care providers in the United States and 14% of the adult population globally, and significantly impacts on health-related quality of life. It may affect individuals at any age but authors say that patients over 65 and women are normally more affected than men. Large expenditures are associated for the diagnostic testing and treatment of this condition and several allopathic and non-allopathic medications can be found in pharmacies and available for the treatment of this condition, however, many of them do not produce desired results [37-39]. It is possible to find in the literature a number of studies with plants that may exert laxative effects and one of them is the well-known senne (*Cassia angustifolia*). The presence of sennosides, anthraquinone, naphthalene, acetophenones, flavonoids, and xanthones in the leaves and pods of this plant may be responsible for the increase in the intestinal motility [1, 40-41]. We did not find studies to compare the effects of MO in the gastrointestinal tract with our results. The chronic use of anthraquinone laxatives such as senne, rhubarb, and aloe capsules are associated to alterations in the colonic epithelial cells leading to a condition of irreversible injury to organelles and resulting in colonic melanosis coli. This condition occurs as a result of an excessive melanin synthesis, and the pigmentation is due to lipofuscin, besides melanin, located in mucosal macrophages [42-43]. Abendroth et al [44] considers that due to pharmaceutical adverse effects of anthraquinone compounds such as electrolytic shift and water loss and increase risk of developing melanosis coli, anthraquinone laxatives should be avoided for long-term therapy of constipation. The laxative effects of MO were studied in an acute experiment (not chronically) and

the pigmentation found the group G2 (Figure 1) probably is associated with pigments present in the plant.

Conclusion

Our results with the use of *Moringa oleifera* flour are very promising, once its use improved lipid profile, prevented weight gain and showed no adverse effects. Thus we may conclude that this flour could be added to industrial products in order to provide healthier products to the consumers. By the other hand, the standardization of products needs further studies.

Conflict of Interests

Authors declare no conflict of interests.

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