BENEFICIAL EFFECTS OF *MORINDA CITRIFOLIA* LINN. (NONI) LEAF EXTRACT ON OBESITY, DYSLIPIDEMIA AND ADIPONECTINEMIA IN RATS WITH METABOLIC SYNDROME

Soto R. Ida 2, Quintana C. Rodolfo 1,2, Rodríguez A. Jorge 4, Xicoténcatl R. Irving 4, Lagunes R. Luicita 3, Aguirre M. Isaac 1 and Alexander A. Alfonso*1,2

Escuela de Medicina 1, Universidad Cristóbal Colón. Boca del Río, Ver., C.P. 94271. México.
CIIDIR-OAXACA 3, Instituto Politécnico Nacional. Santa Cruz, Xoxocotlán, Oaxaca, Oax., C.P. 71230. México.
Centro Tlaxcala de Biología de la Conducta 4, Universidad Autónoma de Tlaxcala. C.P. 90062. México.

INTRODUCTION:

*Morinda citrifolia* Linn. (Rubiaceae), commonly known as “noni,” likely originated in the Indonesian archipelago and has been used for over 2000 years for its medicinal effects; this plant is distributed throughout the tropical and subtropical regions of the world 1.

A wide variety of pharmacological activities have been reported for extracts from *M. citrifolia* Linn. fruit, leaves and roots, however; the plant has not been widely studied for its antidyslipidemic effects with regards to obesity and adiponectin levels in Wistar rats with MS. The sucrose-induced metabolic syndrome model represents the dietary intake of sucrose of humans, *e.g.*, refined sugar in beverages and soft drinks, which is associated with the development of overweight status and obesity.

Obesity is a multifactorial disorder reflecting the complex interactions between genes and

**ABSTRACT:** Pharmacological activities have been reported for the fruit, leaf and root extracts of *Morinda citrifolia* L. (Noni); however, the plant has not been widely studied for its antidyslipidemic effects on obesity and metabolic syndrome (MS). **Objective:** to evaluate the effects of aqueous *M. citrifolia* leaf extract on obesity and adipose tissue cell dynamics associated with triacylglycerol and adiponectin levels in Wistar rats with MS.

**Methodology:** four groups of rats were used; control, metabolic syndrome (MS), *M. citrifolia* (Mc) and *M. citrifolia* plus sucrose (Mc-Suc). The dose of extract was 200 mg/kg of body weight for 2 weeks. **Results:** we observed differences in the weights of abdominal fat between the Mc and MS groups. The Mc-Suc and Mc groups showed significantly lower triacylglycerol concentrations than the MS group. Moreover, the adiponectin serum concentrations were higher in control, Mc-Suc and Mc groups than in the MS group. Finally, the MS group showed an increase in the percentage of fat cells with areas between 1001 and 1501 µm² compared with the Mc and Mc-Suc groups. **Conclusion:** ingestion of the aqueous extract from dried *M. citrifolia* leaves decreases abdominal fat and triacylglycerols associated with increased adiponectin levels and changes to adipose tissue cell dynamics.

**Keywords:** Adiponectinemia, Adipose Tissue, Metabolic Syndrome, *Morinda citrifolia*, Obesity

**Correspondence to Author:**

Professor and Researcher, Facultad de Bioanálisis, Universidad Veracruzana, Veracruz, Ver., C.P. 91700. México.

**Email:** aalexander_2000@yahoo.com

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environment, e.g., lifestyle, and is associated with a high risk of chronic diseases such as diabetes, cardiovascular disease, dyslipidemia, neurological alterations, chronic kidney disease and certain cancers. In 2010, IASO/IOTF analysis estimated that approximately one billion adults were overweight and that 475 million were obese. Patients who are overweight or obese are characterized by the abnormal development of adipose tissue.

Adipose tissue produces a number of bioactive substances (adipokines), including tumor necrosis factor (TNF)-α, leptin, resistin, and adiponectin. The size of individual adipocytes and distribution of sizes within a population have attracted attention with regards to their physiological impacts, and studies have shown that adipocyte size may affect behaviors such as adipokine secretion.

Adiponectin is a 30 kDa protein that is abundantly secreted from adipocytes and circulates as three oligomeric complexes at high concentrations in the blood (3–30 μg/mL). Unlike other adipokines, adiponectin is protective against the development of metabolic disorders, including obesity, insulin resistance, type 2 diabetes and related atherosclerotic vascular disease. In rodent models, deletion of adiponectin is associated with increased inflammatory actions under conditions of stress such as over-nutrition and ischemic insult. The circulating levels of adiponectin are markedly reduced in obese and hypertensive patients and in patients with coronary artery disease, as well as in experimental animal models of insulin resistance and diabetes. Additionally, a number of clinical observations have noted that hypoadiponectinemia is associated with impaired endothelial-dependent vasodilation, hypertension, myocardial infarction, and coronary artery disease. Consequently, the present study aimed to investigate the effects of Morinda citrifolia leaf extract on obesity and changes to adipose tissue cell dynamics that are associated with dyslipidemia and adiponectinemia in rats with sucrose-induced metabolic syndrome.

**MATERIALS AND METHODS:**

**Collection and preparation of plant material:** Dried *M. citrifolia* leaves were collected in San Bartolo Tuxtepec (18°05’35.69” N, 96°06’30.07” W) from the Papaloapan region of Oaxaca, Mexico. Taxonomic identification was performed by the curator of the Herbarium of CIIDIR-OAX, and a sample copy was deposited in their research laboratory for future reference. The plants were washed with tap water, dried on sheets of newspaper for 16 - 20 days, and then pulverized using a mechanical mill to obtain a powder that was subsequently hydrated.

**Preparation of crude extract:** To prepare the crude extracts, 50 g of dried and ground plants was added to 150 mL of distilled water in a flask and allowed to stand for 24 h. The solids were then separated from the liquid using filter paper and then discarded. The solvent was removed using reduced pressure on a rotary evaporator to obtain 15 g of crude extract. The crude extract was then stored in amber colored bottles at 8 °C until use.

**Sucrose-induced metabolic syndrome model:** A total of 20 weaning male Wistar rats (Harlan Teklad), 21 days of age, were individually housed and maintained on a 12-h light/dark cycle at 25 °C. Animal maintenance and handling methods were in accordance with the 1985 National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were divided into two groups: the control group (C; n=5), which received a standard diet, and the metabolic syndrome group (MS; n=15), which received the standard diet plus 40% sucrose, which was added to the drinking water; the animals were fed *ad libitum* for 10 weeks.

**Experimental design:** The animals with induced metabolic syndrome (MS), described above, were divided into two groups. Both groups received a standard diet and *Morinda citrifolia* L. extract (200 mg/kg) for 2 weeks; the first group (Mc-Suc; n=5) received *M. citrifolia* extract and sucrose in the drinking water *ad libitum*, and the second group (Mc; n=5) received *M. citrifolia* extract without sucrose in the drinking water. The metabolic syndrome group (MS; n=5) received 40% sucrose in the drinking water, while the control group (C; n=5) received purified water without sucrose.

At the end of the experimental period, fasted animals were killed by decapitation, and metabolic syndrome parameters were assayed. All animal
experiments were conducted under institutional ethical guidelines.

**Blood Samples:** At the end of treatment period, blood samples from 18-hour-fasted animals were carefully collected to avoid hemolysis. The blood was centrifuged at 1086 X g for 10 min, and serum samples were kept at -20 °C until analysis.

**Analytical Methods:** Serum glucose concentrations were measured using the glucose oxidase method. Total cholesterol and HDL were measured using enzymatic reagents. Triacylglycerols, uric acid, total proteins (PTOT), albumin, and globulin were determined by colorimetric or enzymatic methods using an RA 10,000 Autoanalyzer (Bayer Diagnostics, Tarrytown, NY, USA). Finally, serum adiponectin levels were measured using an ELISA assay for rat adiponectin (Quantikine, R&D Systems).

**Adipocyte Size Measurements:** Random samples of abdominal adipose tissue were fixed in neutral formalin (10% formaldehyde and 0.1 M phosphate buffer, pH 7) for 24 h at room temperature. The samples were embedded in paraffin, and serial 7 mm sections were then cut using a microtome and stained with hematoxylin and eosin. Photomicrographs were obtained at a 40X magnification using an optical microscope (Axioimagen A2, Zeiss) with an Olympus digital camera at a 5.1-megapixel resolution. Adipocyte areas were measured using the AxioVision Real 4.6 software (Zeiss Software, Inc.), and expressed as µm². Areas were measured for adipocytes that were completely enclosed within the field, using five fields per rat. The average adipocyte area was calculated for each rat, and group means were determined from the individual averages for comparisons between the groups.

**Data analysis:** Data are presented as the mean ± SD. Statistical significance was determined by analysis of variance, and Tukey’s multiple range test was used for comparison of means (P < 0.05). The Fisher test was utilized for the relative distribution of adipocyte areas (P < 0.001).

**RESULTS:**

**Experimental Model:** The metabolic syndrome model was generated by administration of drinking water containing 40% sucrose to male Wistar rats for 10 weeks. Table 1 shows a significant difference in the body weight change for rats with (MS group) and without (C group) sucrose ingestion. At 1 week, a moderate increase in body weight was observed for the MS group, but at 5 and 10 weeks, the MS group body weights were significantly higher (P < 0.05) than those of the C group. Table 2 shows the differences in liquid consumption, as well as food and caloric intake, between the two groups (P < 0.05) at 1, 5 and 10 weeks of sugar ingestion.

**TABLE 1: BODY WEIGHTS (g) OF SUCROSE-FED AND CONTROL RATS AT 1, 5 AND 10 WEEKS**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control Group (n=5)</th>
<th>MS Group (n=15)</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81 ± 11  (^a)</td>
<td>120 ± 10  (^b)</td>
<td>39±1</td>
</tr>
<tr>
<td>5</td>
<td>170 ± 11  (^a)</td>
<td>218 ± 13  (^b)</td>
<td>48±2</td>
</tr>
<tr>
<td>10</td>
<td>175 ± 13  (^a)</td>
<td>224 ± 33  (^b)</td>
<td>49±3</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.05 indicates difference compared with the control group

**TABLE 2: LIQUID CONSUMPTION AND FOOD AND CALORIC INTAKE IN SUCROSE- FED (MS GROUP) AND CONTROL (C GROUP) RATS**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (n=5)</th>
<th>MS Group (n=15)</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week(s)</td>
<td>1  (^a)</td>
<td>1  (^b)</td>
<td></td>
</tr>
<tr>
<td>Liquid consumption</td>
<td>38.48 ± 6.21 (^a)</td>
<td>25.36 ± 6.18 (^a)</td>
<td></td>
</tr>
<tr>
<td>Liquid consumption equivalent (kcal)</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Food consumption</td>
<td>30.79 ± 2.76 (^a)</td>
<td>21.83 ± 1.96 (^a)</td>
<td></td>
</tr>
<tr>
<td>Food consumption equivalent (kcal)</td>
<td>117.00 ± 10.48 (^b)</td>
<td>82.95 ± 7.44 (^a)</td>
<td></td>
</tr>
<tr>
<td>Total calories</td>
<td>117.00 ± 10.48 (^a)</td>
<td>82.95 ± 7.44 (^a)</td>
<td></td>
</tr>
</tbody>
</table>

The parameters are expressed in ml/day/100 g bw\(^{-1}\); kcal/day/100 bw\(^{-1}\); and g/day/100 bw\(^{-1}\).

Bw = body weight. \(^a\)P < 0.05 indicates difference from week 1, 5 and 10, compared with the control group.
Effects of *Morinda citrifolia* L. extracts: After the rat sucrose-induced metabolic syndrome model was established, the effects of dietary supplementation with *Morinda citrifolia* L. leaf extract were analyzed. Table 3 shows statistically significant differences between the abdominal fat weights of the Mc and MS groups (8.05 ± 1.43 vs. 15.03 ± 4.80 g; P < 0.05). The Mc-Suc and Mc groups showed significantly lower triacylglycerol concentrations (45% and 68%, respectively, P < 0.05) than the MS group. No differences were observed for the other parameters (Table 4).

### TABLE 3: BODY AND ABDOMINAL FAT WEIGHS OF RATS FED EXPERIMENTAL DIETS FOR 2 WEEKS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (n=5)</th>
<th>MS Group (n=5)</th>
<th>Mc-Suc Group (n=5)</th>
<th>Mc Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>246 ± 16 a</td>
<td>226 ± 14 a</td>
<td>205 ± 48 a</td>
<td>206 ± 17 a</td>
</tr>
<tr>
<td>Abdominal fat weight (g)</td>
<td>3.83 ± 1.92 ab</td>
<td>15.03 ± 4.80 a</td>
<td>16.20 ± 5.95 a</td>
<td>8.05 ± 1.43 b</td>
</tr>
<tr>
<td>Pericardial fat weight (g)</td>
<td>---</td>
<td>---</td>
<td>0.20 ± 0.01</td>
<td>0.60 ± 0.01</td>
</tr>
<tr>
<td>Index of adiposity</td>
<td>1.55</td>
<td>6.65</td>
<td>7.90</td>
<td>3.90</td>
</tr>
</tbody>
</table>

Index of adiposity: (Abdominal fat weight / body weight) x 100

\*abP < 0.05 indicates difference compared with the control group

### TABLE 4: SERUM PARAMETERS IN RATS FED EXPERIMENTAL DIETS PLUS *MORINDA CITRIFOLIA* EXTRACTS FOR 2 WEEKS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Group (n=5)</th>
<th>MS Group (n=5)</th>
<th>Mc-Suc Group (n=5)</th>
<th>Mc Group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>122.33±24.00 b</td>
<td>170.00±37.78 ab</td>
<td>190.67±15.18 a</td>
<td>107.33±24.00 b</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>189.33±38.07 a</td>
<td>145.33±15.89 a</td>
<td>137.33±46.61 a</td>
<td>146.33±47.12 a</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>105.06±15.75 b</td>
<td>255.55±38.33 a</td>
<td>140.88±21.13 b</td>
<td>83.55±12.53 b</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>76.33±7.51 a</td>
<td>63.67±9.29 a</td>
<td>55.67±19.73 a</td>
<td>62.00±25.51 a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.63±1.35 a</td>
<td>3.03±0.49 a</td>
<td>2.63±0.46 a</td>
<td>2.06±0.28 a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.36±0.05 a</td>
<td>5.80±0.26 a</td>
<td>5.20±0.17 a</td>
<td>5.00±1.01 a</td>
</tr>
<tr>
<td>Globulin (mg/dl)</td>
<td>2.76±0.25 a</td>
<td>2.76±0.20 a</td>
<td>2.43±0.40 a</td>
<td>2.40±0.81 a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.68±0.04 a</td>
<td>0.58±0.03 a</td>
<td>0.61±0.01 a</td>
<td>0.69±0.12 a</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>37.66±2.30 ab</td>
<td>27.33±8.62 b</td>
<td>20.66±1.52 b</td>
<td>48.66±12.58 a</td>
</tr>
<tr>
<td>PTOT (g/dl)</td>
<td>8.13±0.31 a</td>
<td>8.56±0.45 a</td>
<td>7.63±0.35 a</td>
<td>7.40±1.83 a</td>
</tr>
</tbody>
</table>

\*abP < 0.05 indicates difference compared with the control group (C group)

As shown in Fig. 1, the adiponectin serum concentration was higher for the C, Mc-Suc and Mc groups than for the MS group. Additionally, abdominal fat weights were negatively correlated with serum adiponectin for the Mc-Suc (r = -0.85), Mc (r = -0.62), MS (r = -0.88) and control groups (r = -0.99) (P <0.05). Finally, Fig. 2 shows the relative distribution of adipocyte areas in samples from abdominal adipose tissues. The SM group displayed a significant increase in the percentage of fat cells, with areas between 1001 and 1501 µm² compared with the group control, and significant differences compared with the Mc and Mc-Suc groups (p < 0.001).

![FIG. 1: SERUM ADIPONECTIN IN RATS FED EXPERIMENTAL DIETS FOR 2 WEEKS](image-url)

*abP <0.05 indicates difference compared with the control group (C group)
DISCUSSION: Our research evaluated the effects of an aqueous extract of dried *Morinda citrifolia* leaves on obesity, dyslipidemia, and adiponectinemia in a Wistar rat animal model of metabolic syndrome. This model was induced by high sucrose intake via drinking water, leading to the development of abdominal obesity, hypertriglyceridemia and hypoadiponectinemia.

These results are consistent with those reported by Aguilera et al., who used similar concentrations of sucrose in drinking water and observed increased abdominal fat, dyslipidemia and high levels of tumor necrosis factor alpha (TNF)\textsuperscript{31}.

With regards to the body weights of rats receiving *M. citrifolia*, Table 3 shows that body weight did not differ among the groups (p < 0.05). Reports in the literature using other animal models, e.g., hamsters, reported that for a high cholesterol diet, there were no changes in body weight upon administration of noni juice\textsuperscript{32, 33}. In contrast, this work shows that rats consuming different dietary energy sources such as sucrose or proteins can have the same body weight but different abdominal fat contents.

The rats that received the aqueous extract of dried of *M. citrifolia* leaves and consumed sucrose (Mc-Suc group) showed equal abdominal fat contents compared to the metabolic syndrome group (MS group); moreover, the group that received the extract without the co-administration of sucrose displayed a significant reduction in abdominal fat compared with the metabolic syndrome group (MS group, p < 0.05). These data indicate that *M. citrifolia* extract inhibits the accumulation of fat; however, the suspension of sucrose intake could also contribute to this result.

It is important to relate these results to the cellular dynamics of adipose tissue prior to the changes in the total weight of body fat, and these results will be discussed later. With regards to serum triglyceride levels, we found that the rats given the *M. citrifolia* extract without sucrose intake displayed a significant decrease compared with the metabolic syndrome group (MS group, P < 0.05).
Similarly, the group of rats given extract and sucrose (Mc-Suc group) showed lower triglyceride levels compared with the metabolic syndrome group (MS group), which showed a significant elevation of these lipids. Similarly, the rats given extract and sucrose (Mc-Suc group) showed lower triglycerides levels compared with the MS group (P < 0.05).

Studies have evaluated the effects of *Morinda citrifolia* on lowering triglycerides in humans as well as in mouse and hamster animal models and observed a significant lowering of triglycerides. Wang *et al.*, 2012 34 reported that noni juice improved lipid profiles and other risk markers in smokers, particularly cholesterol, triglycerides and high sensitivity C-reactive protein. Mandukhail *et al.*, 2010 studied the antidyislipidemic effect of *Morinda citrifolia* (Noni) fruit leaf and root extracts, finding a reduction in triglyceride levels that could be attributed to the plant antioxidant capacity. The active constituents responsible for the antioxidant and antidyislipidemic activities include iridoids 35; several polyphenols belonging to the coumarin 36 and scopoletin 37.

Other proposed antidyislipidemic mechanisms for *Morinda citrifolia* or its active constituents are the inhibition of HGM Co-A, as this enzyme plays a key role in controlling lipid levels in plasma and other tissues 15 and activation of AMPK 38, as its phosphorylation leads to increased glucose uptake and lipid oxidation in muscle cells 39,40.

Finally, concerning the relative distribution of fat cell areas, there was clearly an increase in the percentage of fat cells with areas greater than 1000 µm² caused by the sucrose intake, while the groups receiving the *Morinda citrifolia* extract with and without sucrose intake were similar to the control group.

The above result leads us to hypothesize that the *Morinda citrifolia* extract participates in the cellular dynamics of adipose tissue and therefore the amount of abdominal fat in rodents, tending to reverse metabolic syndrome components.

These results are similar to those of other studies that associated adipocyte size and distribution with several diseases. Size distribution differences were linked with a diabetic phenotype 41, hepatic steatosis 42 and inflammation. Several other studies showed that individual adipocyte sizes may impact behaviors such as adipokine secretion 24. Previous reports have dealt with the issue of size dynamics, examining how adipocyte population size shifts under various dietary conditions and among different rat strains 43,44.

The above result is similar to the results for adiponec tinemia, which was increased in the group receiving *Morinda citrifolia*; these adiponec tinemia levels may contribute to decreased adipocyte inflammation under conditions of stress caused by overnutrition 45, in this case, sucrose intake. Additionally, this study found a negative correlation between abdominal fat weight and adiponec tinemia concentration that could contribute to metabolic syndrome reversal.

CONCLUSION: The ingestion of the *Morinda citrifolia* L. (Noni) dried leaf aqueous extract decreases abdominal fat and serum triglycerides in rats with metabolic syndrome, and this effect is associated with increased adiponec tinemia levels and changes to adipose tissue cell dynamics.

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CONFLICT OF INTEREST: All authors have no conflict of interest regarding this paper.

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