Chronic Supplementation of Noni in Diabetic Type 1-stz Rats: Effects on Glycemic Levels and Exercise Performance

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Abstract

Thirty-two male Wistar rats were used to verify the effects of chronic noni juice administration on blood glucose levels and its relation to physical performance. In half of the rats, diabetes mellitus (DM) was induced with STZ, and the rats were submitted to an incremental workload running test (IWT) until fatigued so that oxygen consumption and performance indexes (exercise time to fatigue and workload) could be analyzed before noni administration. Then, the control and DM groups received a placebo (saline solution) or noni juice (dilution 2:1) at a dose of 2 mL/kg once a day for 60 days. The result was four groups: control + placebo (CP), control + noni (CN), DM + placebo (DMP), and DM + noni (DMN). All groups were then given a third IWT to verify the effect of the noni juice on exercise performance and glycemia. Twenty-four hours after the third test, all animals were euthanized and blood and kidneys were removed for posterior analysis. Noni administration improved the time to fatigue and workload in DM rats by reducing hyperglycemia. These results could be associated with an improved energy efficiency promoted by noni ingestion. However, our results provided evidence that chronic noni administration causes kidney damage since elevated glomerular filtration was observed at the same magnitude as the non-treated DM group. In conclusion, chronic noni ingestion promoted glycemic control and improved the performance in DM rats but caused kidney toxicity.

1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by an absolute or relative deficiency of insulin or its action that promotes hyperglycemia (1). Controlling blood glucose levels within physiological parameters is fundamental to avoiding complications associated with DM. In general, such control is possible by the administration of exogenous insulin or oral drugs, which reduces hyperglycemia events (2).

In this context, the ingestion of certain fruit and herbs seems to contribute to glycemic control through a hypoglycemic effect in DM people. For example, Morinda citrifolia L. (Rubiaceace), popularly known as noni, is a fruit native to Polynesia, Asia, Australia, and the Brazilian coast has been extensively associated with preventing elevated blood glucose levels in diabetics. Its medicinal use has been attributed to a potential antioxidant effect (i.e., ascorbic acid and flavonoids) for the treatment of wounds, infections, menstrual and intestinal irregularities, hypertension, and cancer (3). Specifically, it has been shown that ingestion of fermented noni juice reduced blood glucose levels and increased the use of lipids in diabetic rats (4). Furthermore, noni administration in in vitro muscle cell cultures induced an increase in adenosine 5'-monophosphate-activated protein kinase (AMPK) pathway activation and GLUT4 translocation with increased glucose uptake (5). This response may indicate a possible increase in cellular energy expenditure.

Some results have indicated that fermented noni juice promotes lower glycemia levels, and when taken for medicinal purposes it is diluted with water. This use has produced controversial results (6, 7). In
addition, it has not been determined if chronic intake of noni juice can be toxic. Thus, studies are necessary for clinical confirmation of the fruit and verification of any deleterious effects.

In the field of sports science, some studies have shown a similarity between the mechanisms produced by noni administration and exercise, which promotes glucose uptake as a consequence of AMPK-enhanced GLUT4 translocation, which is insulin independent (8). In addition, it is suggested that the blood glucose level may influence performance during exercise. However, such a result would conflict with studies that the effects of increased glucose availability on performance showed anticipation (9), delay (10) or no effect on fatigue (11).

Therefore, this study aimed to evaluate the effect of chronic noni juice administration on blood glucose levels and its relation to the physical performance of DM rats. In addition, to determine if chronic intake of noni juice is toxic, evaluation was conducted by means of renal analysis.

2. Materials And Methods

2.1. Animals

Thirty-two male Wistar rats weighing about 200 g (6 weeks of age) at the beginning of the study were used. They were acquired from the Central Bioterium ICB/UFMG and habituated to a local bioterium over seven days. They were housed in collective cages under controlled light (0500–1900 hours) and temperature (23.5 ± 1.0°C) conditions with water and rat chow provided ad libitum.

2.2 DM induction

Half of the rats (n = 16) were injected intraperitoneally (i.p) with a single dose of streptozotocin (STZ), 60 mg/kg in 2% solution of 0.1 M citrate buffer. DM was confirmed by polydipsia, polyuria, and glucose levels greater than 300 mg/dL (12, 13). The control group (n = 16) was administrated saline i.p with the same volume. This procedure did not influence the glycemia of the control rats observed in control group.

2.3 Preparation of noni juice in natura

Noni fruit was obtained from the same coastal area of Espírito Santo state, in Brazil. Mature noni was washed; the seeds were removed; and the pulp was put into a blender set on pulse mode. The pulp (in grams) was passed through an extra-fine sieve and diluted in 0.5 mL water at a ratio of 2:1.

The bromatological analysis and bioactive compounds of the noni juice were carried out by a commercial laboratory. The bioactive compounds, phenolics, vitamin C and flavonoids were quantified by enzymatic, gravimetric, gas chromatography and fluorescence methods, according to industry standards.

2.4 Familiarization protocols

After arriving at the laboratory, all rats were introduced to a treadmill designed for small rodents (Modular Treadmill, Columbus Instruments, OH, USA). The familiarization protocol consisted of running on the
treadmill for five consecutive days. The rats were encouraged to run by being given light electrical stimulation (0.5 mA, 0.5 mV) from a grid at the rear of the treadmill belt. Each daily session consisted of running at a constant speed (10 m⋅min\(^{-1}\)) at an inclination of 5% for 5 min. Over the familiarization days, the speed increased gradually and ended at 15 m⋅min\(^{-1}\). This procedure was designed to teach the rats to run and avoid excessive stress during the tests (14).

In the same period, the rats were also familiarized with gavage, the technique chosen for the noni juice administration. This technique guarantees the ingestion of the correct amount established. This familiarization also done to avoid stressing the animals during noni juice administration.

### 2.5 Experimental protocol

After the familiarization protocols, the rats were submitted to the incremental workload running test (IWT) until fatigued to measure three performance indexes: maximal oxygen consumption (VO\(_{2}\)\(_{\text{max}}\)), time to fatigue and workload as performance indexes. Workload was calculated as body weight × exercise intensity × exercise time × treadmill inclination (15). The workload tests began at a speed of 10 m⋅min\(^{-1}\) (5\% inclination) with increments of 1 m⋅min\(^{-1}\) every 3 minutes until fatigue (16, 17). Fatigue was defined as the point when the animals were no longer able to keep pace with the treadmill for 10 s (18). From the result of this test the rats were divided into four balanced groups to guarantee the homogeneity of the metabolic rate among the groups at the beginning of the study. Two of these groups received STZ i.p for DM induction, while the other two were control groups. The DM group was also treated with special-acting insulin (Humulin NPH®, São Paulo, SP): injections of two international units (UI) in the morning at 8:00 and other two UI in the evening at 6:00 (pilot data). In the control rats, the same stress injection solution and attention span were applied. Every three days, capillary glycemia was measured by a drop of blood formed from a small cut performed at the end of the tail. The glycemia was measured through the enzymatic analysis with the glycosimeter (Accu-Chek Performa, Roche Diabetes Care Brasil LTDA, São Paulo, Brazil).

Twenty-four hours after DM confirmation, all rats were submitted to the second IWT to verify its effects on performance and VO\(_{2}\)\(_{\text{max}}\). Following each group was divided into rats that received noni juice or a placebo (water) administered by gavage. Noni juice or placebo was administrated at a dose of 2 mL/kg once a day at 9:00 a.m., for 60 days. The groups were denominated as: control + placebo (CP); control + noni (CN); DM + placebo (DMP); DM + noni (DMN).

After noni administration period, all groups were submitted to a third IWT to verify the effect of noni juice on exercise performance and glycemia. All four groups performed the IWT between 2:00 and 5:00 p.m. to prevent circadian interferences on performance or metabolism. After 24 hours, all animals were euthanized.

### 2.6 Euthanasia
Six hours before the euthanasia, chow was removed from the cages and the animals were left to fast. At approximately 8:00 a.m., the animals were euthanized by decapitation. The blood from the trunk was collected for fasting analysis and the tissues were removed.

2.7 Statistical analysis

The data were reported as mean ± SEM. The normality and homoscedasticity of data distribution were verified using the Ryan–Joiner and Levene test. The differences among the groups were evaluated by a one-way analysis of variance (ANOVA). To evaluate groups and time points, a two-way analysis of variance (ANOVA) followed by Student–Newman–Keuls tests were conducted. The effect size (ES), measured on the Cohen’s d scale was considered for the analysis of data having a coefficient of variation above 30%. ES values were considered trivial (< 0.2), small (0.2–0.5), medium (0.5–0.8), or large (≥ 0.8). Correlations were assessed using Pearson’s coefficient. The significance level was set at $p < 0.05$.

3. Results

3.1 Results of bromatological analysis

The results of the bromatological and bioactive compound analyses of noni juice are demonstrated in Table 1. Data indicated the presence of antioxidant and anti-inflammatory compounds such as flavonoids, phenolic compounds and vitamin C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>0.38 g/100 mL</td>
</tr>
<tr>
<td>Food Fiber</td>
<td>3.63 g/100 mL</td>
</tr>
<tr>
<td>Total lipids</td>
<td>&lt; 0.18 g/100 mL</td>
</tr>
<tr>
<td>Proteins</td>
<td>0.40 g/100 mL</td>
</tr>
<tr>
<td>Calories</td>
<td>3.12 kcal/100 mL</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>0.95 g/100 mL</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>0.017 g/100 mL</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>92.70 mg/L</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>&lt; 0.06 mg/Kg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>50.989 mg/100g</td>
</tr>
</tbody>
</table>

3.2 IWT until fatigue before DM induction
Body weight, and performance and metabolic indexes were measured during the first IWT, and the groups were divided in a balanced way (Table 2). As expected, the data among the groups were not different ($p > 0.05$ for all indexes; Table 2).

### 3.3 Effect of DM induction on IWT

The IWT induced a progressive increase in VO$_2$ in both DMC and DMN groups from the beginning of the exercise ($p < 0.001$). As illustrated in Fig. 1, DM induced a marked decrease in VO$_{2\text{max}}$ in both groups DMP ($64.3 \pm 4.0^\circ\text{C}, 1^\circ\text{ IWT vs. } 59.9 \pm 4.1^\circ\text{C}, 2^\circ\text{ IWT}; p < 0.001, \text{Fig. 1A}$) and DMN ($67.5 \pm 2.8^\circ\text{C}, 1^\circ\text{ IWT vs. } 58.7 \pm 2.9^\circ\text{C}, 2^\circ\text{ IWT}; p < 0.001, \text{Fig. 1B}$). In addition, DM induction reduced by 39% the time to fatigue from the first IWT to the second in the DMP and DMN groups (Fig. 1A and 1B, $p < 0.001$).

### 3.4 Effect of chronic noni juice ingestion on performance indexes

As result of the third IWT, the performance of the control groups CP and CN was lower performance than for the second. This result may have been related to weight gain and growthing of the rats, since body mass of approximately 400 grams were observed. Thus, to compensate for the body mass effect, the workload of the animals was calculated (Table 3).
The DMN group recorded the highest values for the third IWT after noni ingestion compared to the second (Table 3). This improved performance could be also observed through the exercise time (Fig. 2B) and exercise time variation between after and before noni ingestion (DMP – 2.1 ± 5.0 min vs. DMN 2.6 ± 3.6 min; \( p = 0.04 \), Cohen d = 0.53).

Since performance parameters improved after chronic noni administration, the energetic efficiency was analyzed by oxygen consumption as a function of the exercise time percentage. As illustrated in Fig. 3, oxygen consumption between groups DMP and DMN showed no difference, indicating that noni increased energy efficiency over the same time of exercise.

### 3.5 Effect of chronic noni juice ingestion on glucose and triglycerides blood concentration

Chronic noni administration reduced the mean blood glucose calculated by the values obtained during the 60 days (large effect size Cohen's d = 0.86, Fig. 4A). This result may imply in improved glycated hemoglobin values (DMC 13.2% vs. DMN 10.9%).

The diabetic rats showed increased triglyceridemia compared to the control group (CP 147.6 ± 5.5 mg/dL vs. DMP 228.9 ± 21.5 mg/dL, \( p < 0.001 \), Fig. 4B). After diabetic rats were supplemented with noni triglycerides, the values returned to control condition and were lowered compared to those of the DMP group (DMP 228.9 ± 21.5 mg/dL vs. DMN 171.2 ± 14.2 mg/dL, \( p = 0.04 \)).

### 3.6 Effect of chronic noni juice ingestion on kidney morphology

In Fig. 5, noni ingestion produced an important increase in Bowman's space area in the control rats, suggesting glomerular hyperfiltration (CP 0.60 × 106 µm\(^2\) ± 0.02 × 106 µm\(^2\) vs. CN 1.48 × 106 µm\(^2\) ± 0.08 × 106 µm\(^2\); \( p < 0.001 \)). However, in diabetic rats the noni supplementation did not produce additional impairment compared to CN (CN 1.48 × 106 µm\(^2\) ± 0.08 × 106 µm\(^2\) vs. DMP 1.70 × 106 µm\(^2\) ± 0.02 × 106 µm\(^2\) vs. DMN 1.98 × 106 µm\(^2\) ± 0.08 × 106 µm\(^2\)).

### 4 Discussion

The present study showed that chronic noni administration in rats induced an improved mechanical efficiency associated with higher exercise time until fatigue. In addition, noni supplementation produced an antihyperglycemic effect in diabetic rats.

Similarly, other studies have already demonstrated an ergogenic effect after noni administration (7, 19, 20). Shalan et al. (7) observed that four weeks of noni supplementation tripled the swimming effort in rats. This increase in exercise performance was attributed to the peripheral and also to central effects induced by noni.
Centrally, our results demonstrated a reduced relative effort perception in DMN compared to the DMP group (Fig. 3). This fact could be evidenced by similar oxygen consumption values between groups when analyzed at the same relative performance, which was expressed as a percentage of running time. It is worth mentioning that both the time of exercise and workload (Table 3) were higher in the DMN rats. These data suggesting a change in the central modulation that coordinates the motor drive and consequently induces delayed fatigue (21, 22).

Changes in the turnover of neuronal systems could play an important role in the peripheral adaptations associated with the development of fatigue. The Shalan et al. (7) study verified alterations to the central neurotransmitter systems, such as serotonin and dopamine receptors and transporters, associated with fatigue development in rats with noni supplementation. Furthermore, it has already been shown that peripheral signaling integrated in the central brain areas could modifies the effort perception and consequently delays the end of the running time (23). The neuronal 5-HT and DA system profile during exercise could change the running time until fatigue as a result of modifications to the lethargy, rating of perceived exertion and motivation, which interfered with central brain signaling to the active musculature (24, 25). It is important to point out that the experiment in this study was not designed to verify central fatigue. However, the observed peripheral effects could be attributed to both the drive from the central areas and feedback signaling changes.

Peripherally, the results of this study, such as increased energy efficiency in the DMN group (Fig. 4) could have been related to a direct action on central homeostasis neuromodulators, such as serotonin and dopamine, contributing to energetic control to exercise but also a feedback from different muscle and metabolic conditions after noni supplementation. In addition, the DMN group showed a reduced hyperglycemic effect compared to the DMP group (Fig. 5). This result had already been reported by Osman et al. (20) and Shalan et al. (7) during swim exercise protocols and was thought to be the cause of an observed ergogenic effect. It is interesting to note that this study is the first to relate the effects of noni supplementation in rats with diabetes during controlled-intensity exercise performed according to a running protocol until fatigue.

Wang et al. (5) reported improvements in carbohydrate and lipid metabolism via the AMPK pathway in rats supplemented with noni. With specific regard to glucose metabolism, it had already been demonstrated an improved insulin receptor sensitivity beyond an increase in glycogen stores. It has been suggested that noni improves glycogen stores either by increasing glycogen storage, delaying glycogen consumption during exercise or both (20). These data contribute to blood glucose disappear, probably through the improving the carbohydrate muscle and liver uptake.

In the present study, the improved glucose metabolism in DMN rats could have been supported by a reduction in triglyceride plasma concentrations, which could indicate higher lipid oxidation (Fig. 5). This possibility is suggested by the reduction in the relative effort perception in the DMN group associated with a change in substrate use (Fig. 4).
Some studies have associated the ergogenic and metabolic effects of noni administration to the influence antioxidant compounds such as phenols and flavonoids (e.g., epicatechin and catechin) (5, 26, 27). During exercise, there is an increase in the production of oxidative molecules, which may function as intracellular messengers in several physiological processes (28). On the other hand, evidence shows that highly oxidative molecule concentrations represent possible toxicity and damage to the cell that could interfere with the excitation–contraction muscle process and consequently performance (29, 30). The phenolic compounds and flavonoids present in noni have antioxidant and anti-inflammatory characteristics that may contribute to reduce the oxidative status promoting the effects observed on glucose and triglycerides metabolism, beyond the performance in DM rats with noni supplementation.

Although noni supplementation has increased performance and energy efficiency beyond has lowered glycemia and triglyceridemia, it appears to have a potentially toxic effect on the kidneys as demonstrated by morphological analysis (Fig. 5). The increase in the Bowman's space area in rats with noni supplementation (CN and DMN) suggested glomerular hyperfiltration. Our data also demonstrated an increase in Bowman's space with the same magnitude of diabetic impair in control group that ingested noni.

Souza et al. (31) did not observe functional or histological disturbances on the kidneys or liver after nine days of noni juice consumption. Stands out that the juice dilution was lower compared to mentioned in our study (1:10 for the Sousa et al. vs. 2:1 present study). In addition, the period of noni juice consumption in our study was extended (60 days), which may have contributed to the effect on the kidneys.

Noni toxicity appears to be caused by its anti-inflammatory compounds. These impair the function of cycloxygenases and hydroelectrolytics, which then inhibits prostaglandin synthesis leading to chronic renal failure (32). In addition, the potassium content present in noni can negatively impact on kidney function in patients with renal failure (Mueller, 2000).

### 5 Conclusion

Chronic noni administration improved glucose and triglyceride concentration in diabetic rats by increasing performance and energetic efficiency during exercise. However, these benefits should be understood with caution because of possible toxic effects to the kidneys.

### 6 Declarations

**Ethical Approval**

All experimental procedures were approved by the Ethics Committee of the Federal University of Minas Gerais for the Care and Use of Laboratory Animals and were conducted in accordance with the regulations described in the Committee's Guiding Principles Manual (protocol Nº 109/2016).

**Competing interests**
The authors declare that they have no competing interests.

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**Authors’ contributions**

DOF; FGC; JSFB; KJS; JBG collected data with the animals; BPM; MTA; DDS performed additional experiments with diabetic rats that support some methodological choices; MCFC; LAMB; DDS; JBG experiments idealization and academic and technical support; DOF; MVC; LAMB; JBG statistical and graphical analysis; DOF; LAMB; JBG wrote the main manuscript text; all authors reviewed the manuscript.

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**Availability of data and materials**

The datasets analyzed in this study are available from the corresponding author (juliana.guimaraes@uemg.br) upon reasonable request.

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**Figures**
Figure 1

Effect of DM induction on exercise time and VO2max in DMP (Panel A) and DMN (Panel B) rats. White and black symbols represent the values measured in 1° IWT (before DM induction) and 2° IWT (after DM induction), respectively.
Figure 2

Effect of chronic noni administration on exercise time until fatigue and \textit{VO2max} in diabetic rats. Black and gray symbols represent the values measured in the IWT test before and after the 60 days of noni administration, respectively. * represents a difference from the beginning of the exercise. (\(n = 8\) in each group).

Figure 3

Effect of chronic noni administration on exercise time until fatigue and \textit{VO2max} in diabetic rats. Black and gray symbols represent the values measured in IWT test before and after the 60 days of the noni administration, respectively. * represents a difference from the beginning of the exercise. (\(n = 8\) in each group).
Figure 4

Effect of chronic noni administration on glycemia (panel A) and triglyceridemia (panel B). * represents difference from CP. # represents a difference from the DMP group.

Figure 5
Photomicrography of kidney tissue of the control and diabetic rats after chronic placebo or noni administration. Sections stained with H&E; 10x increase in Microscope Nikon.