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Original article

# Anti-obesity effects of tea from *Mangifera indica* L. leaves of the Ubá variety in high-fat diet-induced obese rats

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ABSTRACT

Due to the high content of bioactive compounds, herbal teas are being investigated as adjuvant in chronic disease management. Studies have shown that mango leaf tea contain mangiferin, total phenolics and antioxidants, compounds with many functional properties. Therefore, this study aims to evaluate the anti-obesity effects of tea from *Mangifera indica* L. leaves, Ubá variety (TML), in obese rats fed a high-fat diet (HFD). For this, adult male Wistar rats were divided into three groups (n = 8): the control group (fed AIN-93 diet), obese group (fed a HFD) and treated group (fed a HFD and supplemented with TML for 8 weeks). We analysed biometric measures and serum biochemical parameters of metabolic control, inflammation and oxidative stress biomarkers, histomorphometry of visceral adipose tissue and mRNA expression of peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PPAR-γ), lipoprotein lipase (LPL) and fatty acid synthase (FAS). The consumption of TML (24.7 ± 2.1 mL/day) exerted antioxidant and anti-inflammatory effects, increasing total antioxidant capacity and interleukin-10 serum concentrations, reduced abdominal fat accumulation, upregulated PPAR-γ and LPL and downregulated FAS expression. Our data suggest that TML has therapeutic potential in treating obesity and related diseases through regulating the expression of transcriptional factors and enzymes associated with adipogenesis.



## 1. Introduction

Obesity is currently the largest public health problem in most countries [1]. The speed of increase in occurrence of obesity is disproportionately greater than the development of efficient therapies for the treatment and control of metabolic disorders associated with excessive body adiposity [2]. Clinical studies have demonstrated the anti-obesity potential of teas [3,4]; those derived from *Camellia sinensis* have been the most investigated. Polyphenols, particularly of the catechin group, are the main bioactive compounds present in teas from *C. sinensis*. The *in vitro* anti-obesity effects have been attributed to the polyphenols [5], as demonstrated by animal models [6,7] and human studies [7,8]. The therapeutic effects of tea on body weight reduction and improving metabolic alterations present in obesity are mediated by various mechanisms involving metabolism acceleration [9], reducing inflammation [10], decreasing visceral fat [11] and others through

*Abbreviations:* TML, tea from *Mangifera indica* L. leaves; HDF, high fat diet; PPAR-γ, peroxisome proliferator-activated receptor gamma co-activator 1 alpha; LPL, lipoprotein lipase; FAS, fatty acid synthase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; TAC, total antioxidant capacity; IL-10, interleukin-10; TNF-α, tumour necrosis factor alpha; OGTT, oral glucose tolerance test; AUR, uric acid; CRE, creatinine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein; TG, triacylglycerols; GLU, glucose; HOMA-IR, homeostatic model assessment-insulin resistance index; HOMA-β, homeostasis model assessment of β-cell function.

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modulation of gene expression involved in systemic and adipocyte metabolism [12–15].

Mangiferin, a xanthone that is the major constituent of *Mangifera indica,* is present in both leaves and fruits [16,17]. It reverses dyslipidaemic effects induced by a high-fat diet in mice and hamsters [18,19] and exerts similar effects in humans in addition to promoting reduction of body weight [20]. Studies with high-fat diet-fed rats demonstrated that, in the liver, mangiferin upregulated proteins participating in mitochondrial bioenergetics and downregulated proteins controlling *de novo* lipogenesis, pointing to the potential effect of this xanthone for controlling lipid metabolism and energy homeostasis [2]. Surprisingly, despite knowledge on the therapeutic potential of mangiferin for improving metabolic disorders as mentioned above, which has raised interest in performing studies on mangiferin derivatives aiming to develop pharmacophores [21], there are currently no studies published on the anti-obesity effects of mango leaf tea. In addition to mangiferin, other phenolic compounds have been identified in *M. indica* [22–24], suggesting that tea is a very rich aqueous extract in phytochemicals with interesting biological actions, mediated by anti-inflammatory and antioxidant mechanisms. Mangoes (Ubá variety) grow naturally in some Southeast areas of Brazil, and in previous studies we found that their fruits have much higher mangiferin concentration compared with other varieties (Haden, Tommy Atkins and Palmer) collected from commercial plantations [24]. Recently, we developed in our laboratory one tea from mango leaves of Ubá variety, that contained 0.72 (SD 0.08) mg $mL^{-1}$ of mangiferin concentration, stable up to 48 h after preparation, and it was well accepted by consumers [25].

The present study aims to evaluate anti-obesity effects of the mango leaf tea (Ubá variety) in high-fat diet fed rats. For this, we analysed variables related to biometric measures, metabolic control, inflammatory and oxidative stress, histomorphometry and expression levels of genes, in visceral adipose tissue (peroxisome proliferator-activated receptor gamma co-activator 1 alpha [PPAR-γ], lipoprotein lipase [LPL] and fatty acid synthase [FAS]). Our findings are promising: tea from *M. indica* L. leaves (Ubá variety) has a great potential as an adjuvant therapy in obesity management. Further studies are necessary, focusing on metabolic pathways that can be modulated by the consumption of this tea.

## 2. Materials and methods

### 2.1. Tea preparation

The tea (TML) was prepared with young leaves from *M. indica*, Ubá variety, collected in the Zona da Mata area, Minas Gerais State (Brazil), with an authentication certificate (No.VIC37611) issued by the botanical survey of the Federal University of Viçosa herbarium. The leaves were washed and sanitised with chlorine solution (200 mg $mL^{-1}$), dried at 55 ± 2 °C for 38 h in an oven with air circulation (Marconi MA035, Piracicaba, SP, Brazil) and crushed (Marconi Rotor Mill MA090/CFT, Piracicaba, SP, Brazil) to obtain a fine powder (medicinal plant). The tea was prepared using 50 g of sample added to 1 L of hot water, boiled for 5 min and then filtered (Melitta filter paper N°4). The mangiferin concentration of representative samples of tea, determined by High Performance Liquid Chromatography (HPLC), was (0.717 (SD 0.08) mg $mL^{-1}$); the content of phenolic compounds determined by Folin-Ciocalteu reagent was (1.595 (SD 0.11) mg GAE. $mL^{-1}$); and the radical scavenging activity determined by DPPH test was (80.331 (SD 0.18) %) [25]. The tea was prepared and provided daily to the animals during the experiment.

### 2.2. Experiment, animals and diets

Twenty-four adult male Wistar rats (11 weeks old), supplied by the Animal Laboratory of Biological Science and Health Centre (Federal University of Viçosa, Brazil) were kept under standard conditions, in individual cages with a light/dark cycle of 12 h, humidity of 80 ± 5% and temperature controlled (22 ± 3 °C). All experimental procedures were performed in accordance with the Ethic Committee for Animal Research of Federal University of Viçosa, Brazil (approval registered under the number 34/2013). The experimental diets (Table 1) were determined in the formulation of RESEARCH-DIETS® [26]. Eight animals (control group [CL]) were fed AIN–93 M diet and water *ad libitum,* during the entire experiment. The other 16 animals were fed a high-fat diet (HFD) for obesity induction, for 7 weeks. At the end of this time, body weight gain and fasting glycaemia were evaluated, and the Lee index was calculated to identify the presence of metabolic risk. The overweight animal group was divided into two groups (n = 8): FD (fed a HFD and water) and TT (fed a HFD and supplement daily with 50 mL of TML), for an additional 8 weeks. The distribution of animals ensured similar metabolic conditions in both groups with no statistical difference between the variables: body weight, body weight gain, fasting glycaemia and Lee index. After 15 weeks, animals were submitted to overnight fasting, anaesthetised with isoflurane Cristália 100% (Brazil) and euthanised by cardiac puncture. Blood, abdominal and epididymal adipose tissues were collected and frozen in liquid nitrogen before being stored at −80 °C. Samples of epididymal adipose tissue were prepared for histological analysis.

### 2.3. Analysed parameters

#### 2.3.1. Diet intake, tea consumption and biometric measures

Body weight and food intake were measured daily, and caloric intake was analysed every 24 h, from the eighth week onwards. At the beginning and end of the treatment, the Lee index [27] was calculated using the equation: Lee index = [cube root $\sqrt[3]{}$ body weight (g)/naso-anal length (cm)]. The body weight gain was evaluated each week. After euthanasia, the total fat of the abdominal cavity, including visceral and subcutaneous adipose tissue, was removed and weighed (Gehaka weighing scale BG 2000, Brazil). Visceral fat was defined as the adipose tissue located inside the abdominal cavity and localised between the organs. Abdominal fat was defined as adipose tissue located just behind

**Table 1**
Composition of the experimental diets.

| Ingredients | Diets (g. 100 $g^{-1}$) | | Calories kJ (kcal) | |
|---|---|---|---|---|
| | AIN-93M | HFD | AIN-93M | HFD |
| Casein | 14.00 | 19.50 | 234.30 (56.00) | 326.35 (78.00) |
| Maltodextrin | 15.50 | 10.00 | 259.41 (62.00) | 167.36 (40.00) |
| Saccharose | 10.00 | 34.10 | 167.36 (40.00) | 570.70 (136.40) |
| Corn starch | 46.57 | 5.32 | 779.40 (186.28) | 89.04 (21.28) |
| Soybean oil (mL) | 4.00 | 1.00 | 150.62 (36.00) | 37.66 (9.00) |
| Lard | 0.00 | 20.00 | – | 753.12 (180.00) |
| Cellulose | 5.00 | 5.00 | – | – |
| Mineral mix | 3.50 | 3.50 | – | – |
| Vitamin mix | 1.00 | 1.00 | – | – |
| Bitartarate choline | 0.25 | 0.25 | – | – |
| L-cystine | 0.18 | 0.18 | – | – |
| Cholesterol | 0.00 | 0.15 | – | – |
| BHT | 0.0008 | 0.004 | – | – |
| Total | 100.00 | 100.00 | 1591.18 (380.30) | 1944.30 (464.70) |
| CD (kJ $g^{-1}$) | 15.91 | 19.44 | | |
| CD (kcal $g^{-1}$) | 3.80 | 4.70 | – | – |

BHT: butylated hydroxytoluene; CD: caloric density.

the abdominal wall. Adipose tissues were excised and weighed, and total fat was calculated as total fat% = [excised fat (g)/total body weight (g)] × 100.

*2.3.2. Serum parameters*

After 7 weeks of TML treatment, the oral glucose tolerance test (OGTT) and both homeostatic model assessment-insulin resistance index (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated, according to Matthews, Hosker, Rudenski, Naylor, Treacher and Turner [28]. For this, animals were subjected to fasting for 12 h, and a glucose solution at 40% concentration (2 g of glucose/kg of body weight) was administered by oral gavage. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 min after of the glucose solution administration. Glycaemia was measured using Accutrend GCT-Roche equipment, and insulin was determined using commercial kit (ALPCO, USA). Blood samples obtained during euthanasia were analysed for the following serum parameters: uric acid (AUR), creatinine (CRE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), high density lipoprotein (HDL), triacylglycerols (TG) and glucose (GLU). We used commercial kits (Bioclin Quibasa, SP, Brazil) and BS 200 equipment (Mindray, SP, Brazil) for the analysis. Total antioxidant capacity (TAC) was evaluated by Antioxidant Assay Kit (Sigma-Aldrich, MO, USA). All the analysis was performed following the manufacturer's specifications.

*2.3.3. Tumour necrosis factor alpha (TNF-α) and interleukin (IL)-10 concentrations in plasma*

Concentrations of cytokines TNF-α and IL-10 were determinate in the blood plasma by flow cytometry using the Soluble Protein Master Buffer-BD (CA, USA) kit and the BD-FACS Verse equipment, following the manufacturer's specifications.

*2.3.4. Histological analysis*

Fragments of approximately 5 mm of visceral adipose tissue were fixed (10% formalin) and embedded in paraffin. Sections of 5 μm were laminated and counterstained with hematoxylin-eosin (H&E). The images were obtained using Nikon-Ellipse E600YF-L microscope (Japan). Adiposoft (Fiji) software was used to analyse the area and equivalent diameter and number of adipocytes in adipose tissue [29].

*2.3.5. Gene expression in visceral adipose tissue*

The mRNA expression levels of adipose tissue of four animals (each experimental group) were determinate by the SYBR Green Master Mix kit (Applied Biosystems, CA, USA), following the manufacturer specifications. Total RNA was extracted from 200 μg of adipose tissue of four animals for each sample group using the Trizol reagent (Invitrogen, USA). Total RNA (10 μg) was reverse transcribed to synthesise single-strand cDNA. The cDNA was generated using the M-MV Reverse transcriptase kit for the cDNA synthesis (Invitrogen). The cDNA (2 μL) was subjected to Real-time polymerase chain reaction (PCR) to amplify simultaneously the cDNA fragment of the rat PPAR-γ, FAS and LPL genes, using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a reference gene to normalise the expression levels between samples. The mix for PCR analyses of each sample included 2 μL of previously synthesised cDNA, 5 μL of SYBR Green Mix, 0.4 μL of primer mix (forward primer/reverse primer) and 2.6 μL of Milli-Q (2 ×) purified water (ROCHE, BW, Germany). PCR reactions were performed at 95 °C for 10 min and subjected to 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 for 30 s. A Melt curve was included in the final stage. The analysis was performed in duplicates. The relative expression levels of each gene were determined by the method $2^{-\Delta\Delta Ct}$ on the AB SepOne Real Time PCR System equipment (Applied Biosystem). All oligonucleotides were purchased from Alpha DNA (Canada) (see Table 2).

*2.4. Statistical analysis*

The sample size was calculated based on a within-animals difference for the final weight wanted, adopting a statistical power of 80% according to the method described by Agranonik and Hirakata [30]. Group measurements were expressed as mean and standard deviation (S.D) or mean and standard error (S.E). For parametric data, we used analysis of variance (ANOVA) followed by the *post hoc* Tukey test. For non-parametric data, we used the Kruskal-Wallis test followed by the *post hoc* Dunn's test. Area under curve (AUC) values were calculated using the trapezoidal rule [31]. All statistical tests, performed by SPSS- Statistic V20 software (IBM, Canada), were two-sided. Data with $P < 0.05$ were considered statistically significant.

## 3. Results

*3.1. Diet intake, tea consumption and biometric measures*

TML consumption was 24.7 (SD 2.1) mL/day, maintained constant throughout the treatment. Diet consumption of the CL group was higher ($P < 0.05$) as compared with the FD and TT groups, but there were no significant differences in energy intake. Body weight gain was significantly higher in the FD group in compared with other two groups (CL and TT), and both were statistically equal. No difference was observed in the body mass index (*Lee* index) of the rats from the three groups. The total and visceral adipose tissues were lower in the TT group compared with the FD group, and were statistically equal between the CL and TT groups (Table 3).

*3.2. TML and serum biochemical parameters*

Uric acid and alanine aminotransferase were statistically higher in the FD group and there were no differences between the CL and TT groups (Table 4). Differences in the antioxidant capacity were observed between the experimental groups, being lower in the FD

**Table 2**
Oligonucleotides for mRNA expression study from rat adipose tissue.

| Target gene | Sense initiator (5′- 3′) | Antisense initiator (5′- 3′) | Accession number |
|---|---|---|---|
| PPAR-γ | CATTTCTGCTCCACACTATGAA | CGGGAAGGACTTTATGTATGAG | 537716 – 537717 |
| FAS | AGCCCCTCAAGTGCACAGTG | TGCCAATGTGTTTTCCCTGA | 537710 – 537711 |
| LPL | CAGCTGGGCCTAACTTTGAG | CCTCTCTGCAATCACACGAA | 537712 – 537713 |
| GAPDH | AGGTTGTCTCCTGTCACTTC | CTGTTGCTGTAGCCATATTC | 537675 – 537676 |

PPAR-γ, peroxisome proliferator-activated receptor gamma co-activator 1 alpha; FAS, fatty acid synthase; LPL, lipoprotein lipase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. All oligonucleotides were purchased from Alpha DNA (Canada).

**Table 3**
Effects of TML treatment on physiological parameters of rats fed with HFD.

| | CL | | FD | | TT | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Tea consumption (mL/day/animal) | – | | – | | 24.7 | 2.1 |
| Diet consumption (g/day/animal) | 19.3 | 2.4[a] | 15.4 | 1.6[b] | 14.1 | 1.1[b] |
| Caloric intake (kcal/day/animal) | 73.3 | 9.2 | 71.0 | 7.2 | 64.7 | 4.6 |
| Initial body weight (g) | 371.7 | 20.0[b] | 413.6 | 32.3[a] | 423.3 | 29.5[a] |
| Final body weight (g) | 435.7 | 21.7[b] | 503.1 | 35.3[a] | 469.6 | 50.1[b] |
| Weight gain | 64.0 | 19.5[b] | 89.5 | 17.5[a] | 44.0 | 18.4[b] |
| Initial *Lee* index | 2.5 | 0.1 | 2.6 | 0.1 | 2.5 | 0.1 |
| Final *Lee* index | 2.6 | 0.1 | 2.6 | 0.1 | 2.6 | 0.1 |
| Adipose tissue weight (g) | | | | | | |
| Total | 20.6 | 9.1[b] | 44.4 | 13.6[a] | 25.7 | 8.7[b] |
| Visceral | 0.4 | 0.1[b] | 0.7 | 0.1[a] | 0.5 | 0.1[b] |

CL, AIN93 diet; FD, high-fat diet; TT, high-fat diet with TML treatment. [a,b]$p < 0.05$. Significant differences between experimental groups by Tukey test (n = 8).

group. The fasting glycaemia was lower in the TT group, indicating that the TML affected glucose metabolism. No differences were found in the other parameters (Table 4).

### 3.3. Effect of TML on glucose metabolism

During the OGTT, the TT group exhibited significantly higher glucose inflow, which promotes insulin function, as compared with the other groups (Fig. 1a). The AUC values are presented in Fig. 1b. No significant difference was observed in the HOMA-IR index (Fig. 1c). As illustrated in Fig. 1d, the TML stimulates β-cell function (TT: 158.1 (SD 12.5)), which was higher as compared with (CL: 98.3 (SD 7.3)) and (FD: 82.9 (SD 3.7)) groups.

### 3.4. Effect of TML on anti- and pro-inflammatory parameters

TNF-α and IL-10 plasma concentrations are shown in Fig. 2. The FD group presented lower (33.5 (SD 2.3) pg $mL^{-1}$) and higher (121.1 (SD 21.2) pg $mL^{-1}$) concentrations of IL-10 and TNF-α, respectively, when compared with the CL and TT groups. No significant differences in TNF-α concentration were found between the CL and TT groups (Fig. 2b). Although the IL-10 concentration in the TT group was not statistically different between the other two groups, there was a tendency to increase compared with the FD group (Fig. 2a).

### 3.5. Effect of TML on morphometric parameters in visceral tissue

In Fig. 3, the images illustrate the effect of a high-fat diet and TML supplementation on the visceral adipose tissue (Fig. 3a–c). The TT group presented morphometric parameters statistically equal to the CL group, indicating that the TML reversed the effects induced by the HFD diet (Fig. 3d–f). A larger area of adipocytes was observed in the FD group (3738.2 (SD 288.9) $\mu m^2$) compared with the CL (1624.7 (SD 98.6) $\mu m^2$) and TT (2103.8 (SD 390.6) $\mu m^2$) groups, demonstrating that the HFD diet caused hypertrophy of adipocytes (Fig. 3d). The equivalent diameter of adipocytes was significantly lower in the CL and TT groups (Fig. 3e) and was not different between them. Adipocyte number was lower in the FD (45.7 (SD 3.7)) group compared with CL (77.4 (SD 5.6)) and TT (86.1 (SD 15.3)) groups (Fig. 3d).

### 3.6. Effects of TML on gene expression of visceral adipose tissue

Fig. 4 show the mRNA expression levels of PPAR-γ, FAS and LPL in the three groups. PPAR-γ expression was downregulated in the FD group, which was reversed with TT treatment. The TML downregulated the FAS expression in the TT group. The LPL expression was upregulated in the TT group (1.5 and 2.2 times) in comparison with the CL and FD groups, respectively. Thus, this result demonstrated that the TML increased the mRNA level expression of PPAR-γ and LPL and decreased FAS expression after 8 weeks of treatment.

## 4. Discussion

The Ubá mango is one variety that grows in Brazil, and it is organically cultivated through simplified management techniques, enabling their own natural defence against biotic and abiotic stresses. In this condition, it is believed to stimulate the synthesis of phenolic compounds. Therefore, the Ubá mango is one variety with great potential to supply a high quantity of functional compounds from fruits, leaves and bark. Here, we report the first study on the anti-obesity effects of tea from *M. indica* leaves of the Ubá variety (TML) in rats fed a high-fat diet.

As expected, we observed higher body weight gain and accumulation of the visceral and total adipose tissue in the animal group fed a high-fat diet, in comparison with the control group. During 8 weeks of treatment, the TML reduced both body weight gain and deposition of visceral fat without affecting food intake.

The high-fat diet increased the uric acid and decreased the antioxidant capacity in the FD group, and the TML reversed these

**Table 4**
Effects of TML on serum biochemical parameters of rats fed with HFD, after 8 weeks of treatment.

| | CL | | FD | | TT | |
|---|---|---|---|---|---|---|
| | Mean (S.D) | Median (IR) | Mean (S.D) | Median (IR) | Mean (S.D) | Median (IR) |
| AUR, mg/dL | 1.5 (0.4)[b] | 1.3 (0.8) | 2.5 (0.2)[a] | 2.6 (0.3) | 2.0 (0.4)[b] | 2.1 (0.8) |
| CRE, mg/dL | 0.6 (0.1) | 0.6 (0.2) | 0.6 (0.1) | 0.6 (0.2) | 0.7 (0.1) | 0.7 (0.1) |
| AST (U/L) | 150.4 (47.4) | 128.0 (78.0) | 158.4 (51.7) | 141.0 (69.0) | 148.3 (17.0) | 148.0 (28.0) |
| ALT (U/L) | 47.2 (8.0)[b] | 45.0 (13.0) | 224.8 (78.7)[a] | 224.0 (149.0) | 42.4 (5.9)[b] | 45.0 (12.0) |
| Total cholesterol, mg/dL | 68.2 (8.4) | 65.5 (17.0) | 71.2 (12.3) | 68.5 (26.0) | 59.0 (7.9)Submit Final Article | 58.0 (10.0) |
| HDL-cholesterol, mg/dL | 23.2 (1.3) | 23.0 (3.0) | 21.8 (4.6) | 21.5 (10.0) | 22.0 (0.7) | 22.0 (1.0) |
| Triacylglycerol, mg/dL | 64.4 (9.8) | 63.0 (19.0) | 74.0 (22.3) | 80.0 (44.0) | 55.2 (16.7) | 49.0 (32.0) |
| Fasting glycaemia, mg/dL | 118.3 (7.8)[a] | 120.5 (13.7) | 132.0 (12.7)[a] | 132.5 (24.5) | 100.5 (0.6)[b] | 100.5 (1.0) |
| Fasting insulin, μIU/mL | 19.2 (6.5) | 17.4 (11.5) | 15.7 (1.8) | 16.1 (3.2) | 16.4 (2.4) | 16.2 (4.6) |
| Antioxidant capacity m M Trolox | 0.6 (0.0)[a] | 0.6 (0.0) | 0.4 (0.0)[c] | 0.4 (0.1) | 0.5 (0.1)[b] | 0.5 (0.2) |

AUR, uric acid; CRE, creatinine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL, high density lipoprotein; CL, AIN93 diet; FD, high-fat diet; TT, high-fat diet with TML treatment. Values are expressed as the means (standard deviation) and median (interquartile range) (n = 8). [a–c]$p < 0.05$. Significant differences between experimental groups (Kruskal-Wallis, post hoc of Dunn's).

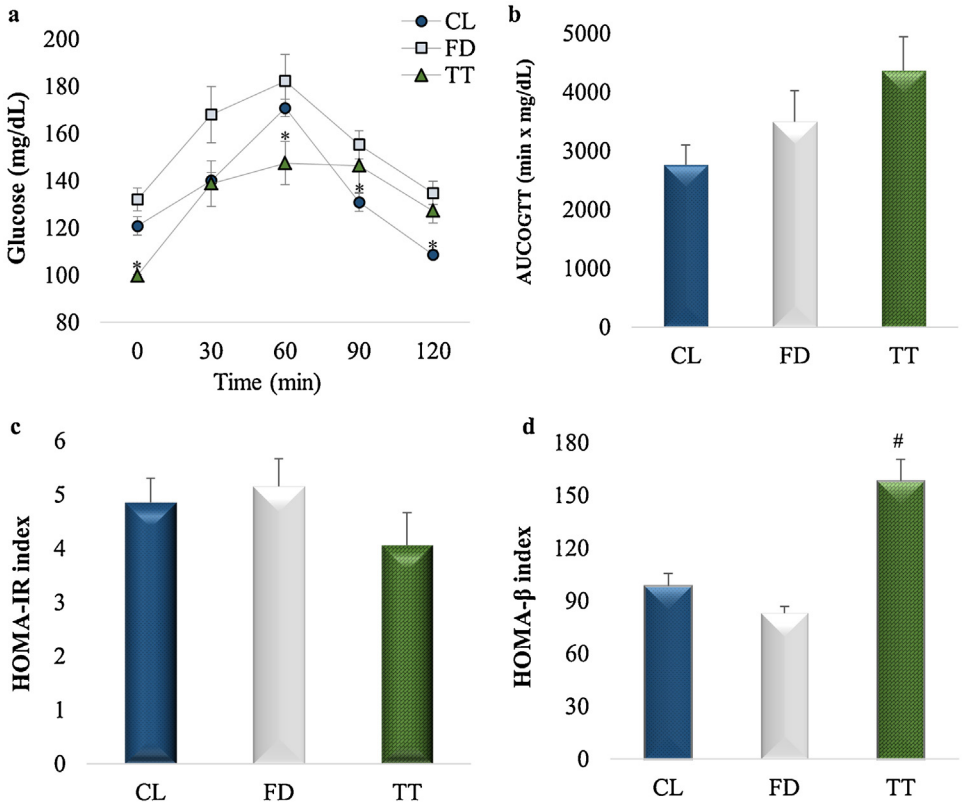


**Fig. 1.** (a) Oral glucose tolerance test, (b) area under curve (AUC) for blood glucose, (c) homeostatic model assessment-insulin resistance index (HOMA-IR) and (d) homeostasis model assessment of β-cell function (HOMA-β). CL, AIN93 diet; FD, high-fat diet; TT, high-fat diet with TML treatment. Values are expressed as means and S.E (n = 8). *Indicates a significant difference between equal times in the different experimental groups. #Indicates a significant difference between the groups. Kruskal–Wallis test, post hoc of Dunn's ($p < 0.05$).

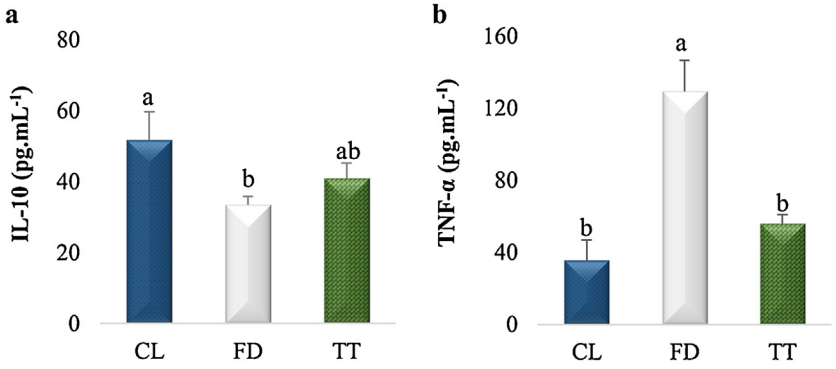


**Fig. 2.** Serum concentrations (pg $mL^{-1}$) of (a) interleukin-10 and (b) tumour necrosis factor alpha in animal groups: CL, AIN93 diet; FD, high-fat diet; TT, high-fat diet with TML treatment. Values are means and S.E (n = 8). $^{a,b}p < 0.05$, significant differences between experimental groups by Tukey test.

alterations. Elevated consumption of fats and sugars leads to obesity, and in this condition the renal excretion of serum uric acid is impaired. The AUR reduction in the group with TML supplementation may be related to the lower body weight gain [32].

The TML did not affect the creatinine concentration, a serum marker of kidney function, indicating that the tea did not exert toxic effects on the kidney [33,34]. ALT, a glucogenic enzyme synthesised in liver, is considered a serum marker of hepatic function and its elevation is indicative of insulin resistance induction and hepatic damage [35]. In the present study, the TML decreased the ALT concentration that had been elevated by a high-fat diet, returning it to a physiological level similar to that of the control group.

The glycaemic curve profile in the group treated with TML showed a higher uptake of postprandial glucose in the 30–120 min interval. These results indicate that the TML stimulated glucose uptake and then improved postprandial glucose metabolism. The high-fat diet and TML did not affect the HOMA-IR index. Lower values of the HOMA-β index were observed in the high-fat diet group, suggesting an insufficient insulin secretion, associated with type II diabetes and insulin resistance risk [36–38]. Our data showed that the TT group had higher glucose inflow and greater HOMA-β index value, compared with the other groups, suggesting that the TML delayed β-cell apoptosis and improved insulin secretion.

Evidence highlights the contribution of adipose tissue to a systemic inflammatory state associated with increased levels of

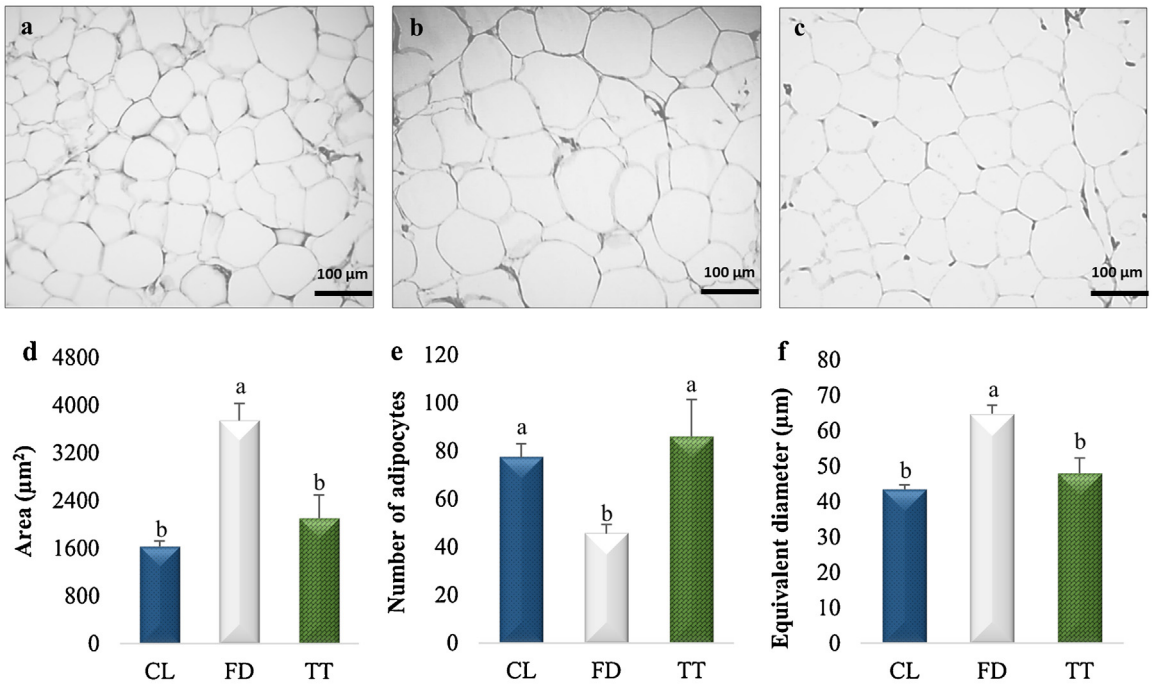


**Fig. 3.** Histomorphometric analysis of visceral adipose tissue in the animal groups. (a) AIN93 diet [CL], (b) high-fat diet [FD]; (c) high-fat diet with TML treatment [TT]. Histomorphometric measurements (d) adipocyte size, (e) number of adipocytes and (f) equivalent diameter. Values are means and S.E (n = 8). $^{a,b}p < 0.05$, significant differences between experimental groups (Kruskal–Wallis, post hoc of Dunn's).

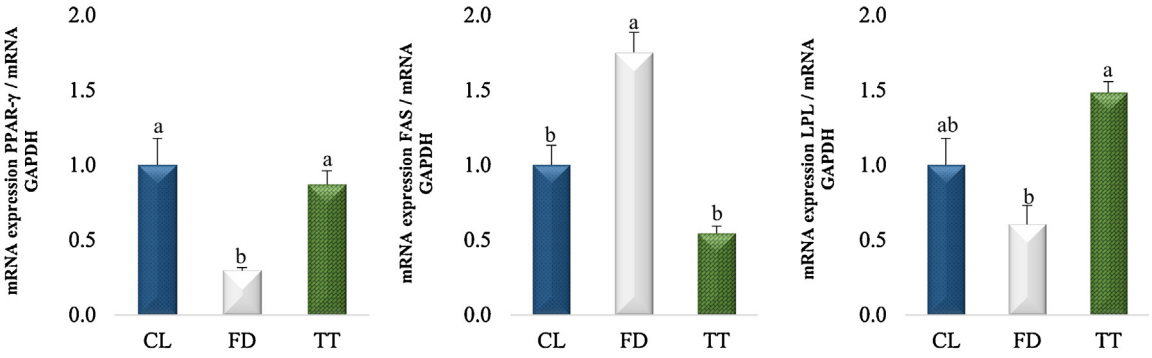


**Fig. 4.** Effect of TML on mRNA expression levels of PPAR-γ, LPL and FAS in visceral adipose tissue by PCR analysis. Gene expression was expressed in arbitrary units based on the calculated expression level relative to the internal standard (n = 4). Values are means and S.E. $^{a,b}p < 0.05$, significant differences between experimental groups, ANOVA followed by the *post hoc* Tukey test. CL, AIN93 diet; FD, high-fat diet; TT, high-fat diet with TML treatment.

pro- inflammatory cytokines, such as TNF-α [39] and decreased levels of anti-inflammatory cytokines such as IL-10 [40]. Excessive adiposity stimulates secretion of proinflammatory cytokines. TNFα is a major player mediating the activation of signalling cascades in adipocytes that are central to inflammation and insulin resistance [41]. IL-10 is produced by a wide range of immunological cell types, including monocytes and macrophages. It is a potent inhibitor of proinflammatory cytokines and chemokines [42], and prevents diet-induced insulin resistance [43]. The TML exerted anti-inflammatory effects by increasing IL-10 and decreasing TNF-α concentrations. This effect attenuated the proinflammatory state induced by a high-fat diet.

At the systemic level, in our study, we observed that a high-fat diet induced a drastic increase of ALT, decrease of TAC and stimulation of the proinflammatory state, with elevation of TNF-α and reduction of IL-10. These results have coherence because, in response to an inflammatory stimulus, an increased migration and infiltration of macrophages may occur in pancreatic islets, liver, and adipose tissue with cell and tissue malfunction, leading to reduce insulin secretion [44]. The TML reversed the metabolic disturbances high-fat induced and normalized blood glucose and insulin through anti-inflammatory and antioxidant effects.

The TML limited the adipose tissue expansion that was induced by the high-fat diet. This tea decreased the adipose cell size and increased its number, which morphologically characterised the hyperplasia [45,46]. Other authors have reported that a high-fat diet induces hypertrophy in animal visceral adipose tissue [47,48].

To explore which genes could be modulated by TML, we evaluated the mRNA expression levels of PPAR-γ, LPL and FAS. The TML affected the mRNA expression of these genes. The high-fat diet downregulated PPAR-γ, and the TML reversed this effect, upregulating PPAR-γ to that of the control group. Fatty acid synthase was downregulated, and lipase lipoprotein was upregulated, indicating a significant improvement in fat metabolism in adipocytes. PPAR-γ is the central regulator of adipose cell differentiation and development [49]. PPAR-$\gamma$ acts synergistically with others transcriptional regulators to govern adipogenesis through a tightly controlled transcriptional cascade [50].

FAS is over-transcribed and over-expressed in adipose tissue in rats and humans with obesity [51,52], and catalyses the *de novo* lipogenesis of long-chain fatty acids. FAS inhibitors lead to dramatic body weight loss in mice [53] and therefore this enzyme has been considered as an anti-obesity target [54]. Gene expression of FAS was lower with TML treatment, suggesting that the effect on reducing adipose tissue could be due to its inhibitory effect on *de novo* lipogenesis.

LPL is an enzyme that allows adipose tissue to take up free fatty acids from triglycerides circulating in lipoproteins [55] and thus can improve glucose metabolism in obesity [56].

Together, our findings demonstrate that the TML has potential benefits in mitigating adipose inflammation, oxidative stress and impaired glucose metabolism induced by a high-fat diet. In addition, it inhibited the expression FAS, a lipogenic gene, and significantly up-regulated the expression of fatty acid oxidative gene (PPAR-γ). Furthermore, the stimulation of LPL expression improved systemic metabolism, frequently dysregulated in obesity. The modulation of these genes can explain part of the action mechanisms of TML on the histomorphometric parameters in visceral adipose tissue. TML is very rich in polyphenols and it has been reported that these compounds can decrease fat accumulation and generation of reactive oxygen species in adipocytes, through reactive oxygen species-mediated down-regulation of PPAR-γ and the mRNA expression downregulation of FAS [57].

Taken together, these results indicate that anti-obesity effects of TML occur by means of PPAR-$\gamma$ activation, improving adipose tissue metabolism, which has repercussions in reducing inflammation and oxidative status at the systemic level.

## 5. Conclusion

Our study demonstrated the effect of TML on the fat mass accumulation, improving biometric and metabolic features associated with a high-fat diet. Morphometry of visceral adipose tissue was modified by the tea from mango (*M. indica*) leaves, with reduction of adipocyte size. There was improvement in the adipose tissue metabolism through up-regulation of PPAR-$\gamma$ and LPL and downregulation of FAS. The modulation of PPAR-$\gamma$ by TML is relevant and demonstrates its potential for clinical applications in obesity and associated metabolic diseases.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Ethical standards

The experimental procedures were performed in accordance with the Ethic Committee for Animal Research of Federal University of Viçosa, Brazil (approval registered under the number 34/2013). The manuscript does not contain clinical studies or patient data.

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