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Angiotensin II type 1 receptor (AT1) blockade by Telmisartan attenuates hepatic steatosis in high-fat fed mice reducing Resistin, TRL4, and Myd88 expression

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Abstract

Background: Telmisartan is a non-peptide angiotensin II receptor antagonist which acts by ACE/AngII/AT1 axis blockade (ARB). In the last years increasing evidence of its metabolic benefits pointed out this drug as the most promising ARB for nonalcoholic fatty liver disease (NAFLD) treatment. The aim of the present study was to investigate the Telmisartan effect on treating NAFLD in mice fed with a high-fat diet evaluating liver gene modulation. Twenty-four male mice were divided into four groups and fed for 60 days with a standard diet (ST), standard diet plus TEL (ST+TEL 5 mg/kg/day by gavage for 4 weeks), high-fat diet (HFD), or high-fat diet plus TEL (HFD+TEL 5 mg/kg/day by gavage for 4 weeks). Body weight, lipid profile, insulin, alanine transaminase, and aspartate aminotransferase were evaluated. Liver histology was analyzed. US imaging was performed to access liver dimension and echogenicity and also epididymal fat pad thickness. The expression of proinflammatory resistin/TRL4/MYD88 pathway was analyzed.

Results: The main findings showed that TEL reduced the resistin, TRL4, and Myd88 liver expression in the HFD + TEL group when compared to the obese control group (HFD). Decreased hepatic steatosis in the HFD + TEL group demonstrated by US measurements of the liver longitudinal axis and echogenicity were observed. In addition, TEL reduced epididymal adipose pad thickness, body weight, transaminases, and improved glucose tolerance test and HDL cholesterol.

Conclusions: We observed that Telmisartan treatment improved metabolism, decreasing NAFLD.

Keywords: Renin-angiotensin system, Obesity, Angiotensin-(1–7), Non-alcoholic fatty liver disease

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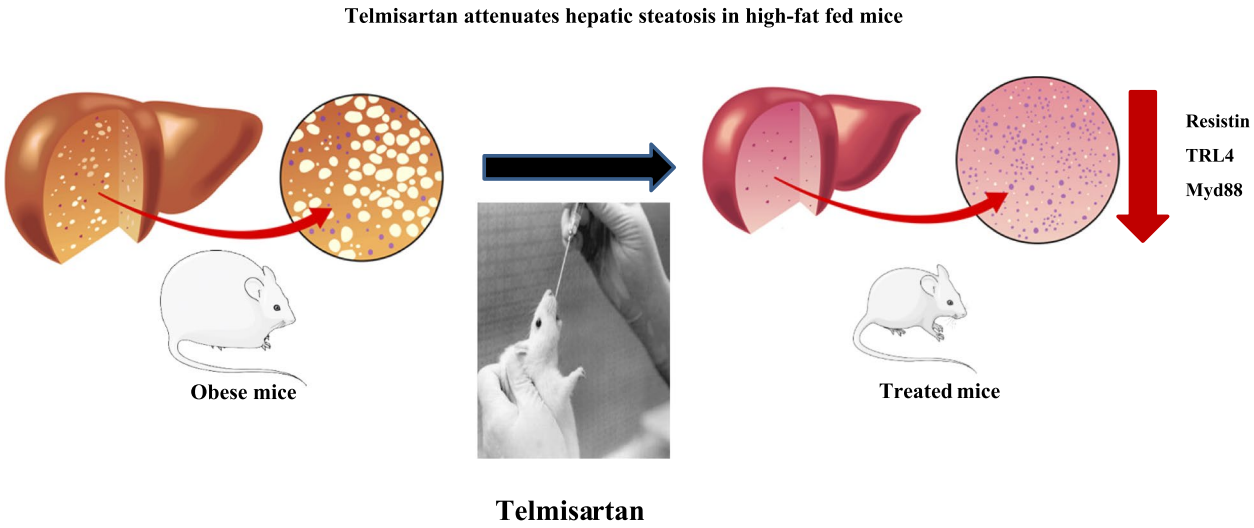
Graphical Abstract

Telmisartan improves metabolic and lipid profile and liver steatosis of obese mice



Background

The diet and lifestyle changes in the last decades culminated in a worldwide increase in the metabolic syndrome (MetS) prevalence; a complex disorder associated with multiple clinical components such as obesity, hypertension, dyslipidemia, glucose intolerance, and insulin resistance (type 2 diabetes) [1]. Non-alcoholic fatty liver disease (NAFLD) is now considered the hepatic component of MetS since both conditions share the same risk factors [2]. With a global prevalence estimated to be 25.2% of the total population and with wide geographical variation across the world [3, 4], NAFLD stands out as one of the main causes of chronic liver pathology and is defined as a fatty hepatocyte infiltration, excluding alcohol abuse situations (women ≤20 g/d, men ≤30 g/d). The excessive fat culminates in increased oxidative stress, which starts a cycle of injury, inflammation, and fibrosis in the hepatic parenchyma. Some patients reach the most serious spectrum of the disease, with nonalcoholic steatohepatitis (NASH) and/or liver cirrhosis and its clinical consequences, including carcinogenesis [5].

As a clinically silent condition and with nonspecific laboratory markers, NAFLD diagnosis is imaging-based or by histological techniques [6, 7]. The NAFLD pharmacotherapy priority is to improve the disease pathophysiology preventing the NAFLD evolution into NASH the most serious form of liver disease [8]. Thus, telmisartan has been shown to be a powerful ally for this goal, demonstrating positive results in hepatic steatosis, insulin resistance, inflammation, and fibrosis [9, 10].

Telmisartan (TEL) is one of the main drugs widely used for the systemic arterial hypertension treatment improving the hypertension-related cardiovascular end-organ damage. TEL acts as an angiotensin type I receptor blocker (ARB) and presents excellent tolerance and safety [11]. Recent studies highlighted possible TEL metabolic effects improving MetS parameters and patients' glycemic control [12]. Animal studies also demonstrate promising hepatoprotective effects with this ARB against fat infiltration and inflammation of liver parenchyma [13].

Toll-like receptors (TRLs) are a family of pattern recognition receptors, being identified as 11 TLRs in humans with several ligands [14]. TRL receptors participate in the defense against many pathogens and play a crucial role in the innate immune system function by activating NF-kB and other signaling pathways to produce inflammatory cytokines. The TLR4 is the most studied receptor [15, 16], and its intracellular signaling produces the complex TLR4/myeloid differentiation factor 2 (MD2) through human myeloid differentiation factor 88 (MyD88) resulting in the migration of nuclear factor-κB (NF-κB) transcription factor [17], which modulates inflammation.

Another important proinflammatory molecule is the resistin, which is now considered a protein "found in the inflammatory zone" (FIZZ), that actively participates in the inflammation process by inducing and responding to several stimuli-like cytokines, mitogens, and endotoxins [18]. Its levels are increased in some conditions such as chronic liver diseases, obesity [19], lung diseases [20], atherosclerotic plaques [9], arthritis [21], and kidney

disease [22]. It has been demonstrated strong correlation of resistin with other inflammatory markers such as C-reactive protein, tumor necrosing factor (TNF-α) [23], interleukin-6 (IL-6), and soluble vascular adhesion molecules, so its reduction has been faced as a promising predictive tool in chronic diseases [10].

In this context, the present study aimed to investigate the TEL treatment ability to improve NAFLD in high-fat fed mice evaluating liver anti-inflammatory effects associated with gene expression.

Material and methods

Animals

The experiment was conducted with 24 male FVB/N mice (4 weeks old), which were randomly divided into four groups ($n = 6$) and fed with the following experimental diets for 4 weeks with control diets or a high-fat diet and then treated for additional 4 weeks with TEL 5 mg/kg/d by gavage being divided as follows: standard diet (ST), standard diet plus TEL (ST+ TEL), high-fat diet (HFD), and high-fat diet plus TEL (HFD+ TEL). Metabolic effects of TEL have been demonstrated especially when it was given at doses ≥ 5 $mg \cdot kg_{bw}^{-1} \cdot day^{-1}$ [24]. This study was approved by the Ethics Committee of Experimentation and Animal Welfare of Unimontes, Montes Claros, Brazil (process no. 022/2012).

Diets

The control group animals were fed with a diet that presented an energetic value of 2.18 Kcal per 1 g of diet, containing the following components: 50.30% carbohydrate, 41.90% protein, and 7.80% fat (Labina, Purina, St. Louis, MO, USA) [25]. The high-fat diet was composed of 24.55% of carbohydrates, 14.47% of proteins, and 60.98% of fats, presenting a total of 5.28 kcal per 1 g of diet [26]. All the high-fat diet components were purchased from Rhoster LTDA (São Paulo, Brazil).

Measurements of body weight, food intake, and tissue collection

The mice were individually housed and the food intake was measured twice per week during treatment to determine food efficiency (food intake/body weight). Overnight-fasted mice were sacrificed with ketamine (130 mg/kg) and xylazine (0.3 mg/kg) after anesthesia [25, 26]; sample tissues were collected, weighted, and immediately frozen in liquid nitrogen and stored at −80 °C for posterior analysis.

Ultrasound imaging (US)

Animals were studied in a supine position, on the day before the sacrifice, by the same trained radiologist, using Medison® ultrasound equipment (Seoul, South Korea), with a multifrequency linear transducer (7.0 to 12 MHz). All imaging was performed in fundamental brightness mode (B mode), with optimization of the gain and the time gain compensation settings, which were kept constant throughout the experiment. The acoustic focus was placed in the center of the target organs (liver and epididymal fat pad), with the measurement of liver echogenicity, size (longitudinal axis), and epididymal fat pad thickness. Liver echogenicity was analyzed using the public domain Java image processing program ImageJ (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, Maryland, USA). A region of interest (ROI) was determined arbitrarily in the constant area of ultrasound images of the liver parenchyma, covering a wide area and excluding large vessels as far as possible. The average intensity in the ROI was measured and relative values were compared.

Determination of blood measurements

The animal's lipid and hepatic profiles were analyzed by measuring the serum levels of total cholesterol, high-density lipoprotein (HDL), triacylglycerol, and aspartate and alanine transaminases (AST and ALT). For the aforementioned measurements, serum was obtained after blood centrifugation at 3200 rpm for 10 min at 4 °C. All analyzes were performed using specific enzymatic kits (Wiener Laboratories, Rosario, Argentina).

Hematoxylin and eosin staining

Liver samples were fixed in formaldehyde solution (10%) and embedded in paraffin serially sectioned at 5 mm, stained with hematoxylin and eosin (HE), and evaluated under a conventional light microscope using an Olympus BX50 microscope (Tokyo, Japan) under the 20 × optical magnification. For each slide, images from the three most affected fields (×20 objective lenses) were considered. Biopsy analyses were classified according to Hübscher (2006) depending on fat accumulation [27].

Analysis of proinflammatory gene expression in the liver

In order to verify the anti-inflammatory effects of TEL in NAFLD, specific liver inflammatory process genes were evaluated. Hepatic tissue fragments were used to obtain total RNA using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The total RNA obtained was treated with DNAse (Promega), and the RNA transcription into cDNA was obtained via reverse transcription M-MLV (Invitrogen Corp.) using random hexamer primers.

Gene expression was normalized to endogenous glyceraldehydes-3-phosphate dehydrogenase (GAPDH). The mRNA levels of the genes were determined by qRT-PCR and amplified using specific primers and reagent SYBR green (Applied Biosystems, USA) on 384-well Quant Studio 6 flex equipment (Applied Biosystems). The primer sequences are detailed in Table 1.

Table 1 Primers sequences used for Real-time PCR analysis

Gene	Forward primer	Reverse primer
GAPDH (NM_011146)	5′AAGAAGGTGGTGAAGCAGGCATC3′	5′CGAAGGTGGAAGAGTGGGAGTTG3′
TRL4 (NM_007678)	5′TTCAGCTCTGGGATGACCTT3′	5′GCCGTTAGTGAAGAGTCTCAGTTTG3′
RESISTIN (NM_007643)	5′TAGTAGAACCGGCCACGTA3′	5′CAGTTCCGATCACAGCCCAT3′
MYD88 (NM_009127)	5′CATCGCCTGCTCTACCCTTT3′	5′GAACTGCGCTTGGAAACCTG3′

For the relative quantification, the standard dietary group (control group) was employed as a calibrator. The results were quantified as cycle threshold (Ct) values, where Ct was defined as the PCR threshold cycle in which the amplified product is first detected and defined as relative gene expression (the target/endogenous ratio). The relative gene expression was analyzed by the $2\Delta\Delta^{Ct}$ method [28].

Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA) and analyzed with confidence 95% and considered $P < 0.05$ was statistically significant. All the data reported in the present study are presented as the mean ± standard deviation (SD) and analyzed via one-way ANOVA followed by Bonferroni post-test.

Results

Mice fed with HFD had a higher energy intake than those fed with an STD diet, and treatment with TEL did not reduce their energy intake (ST:2.69 ± 0.07; ST+TEL:2.40 ± 0.05; HFD:3.20 ± 0.13; HFD+TEL:3.25

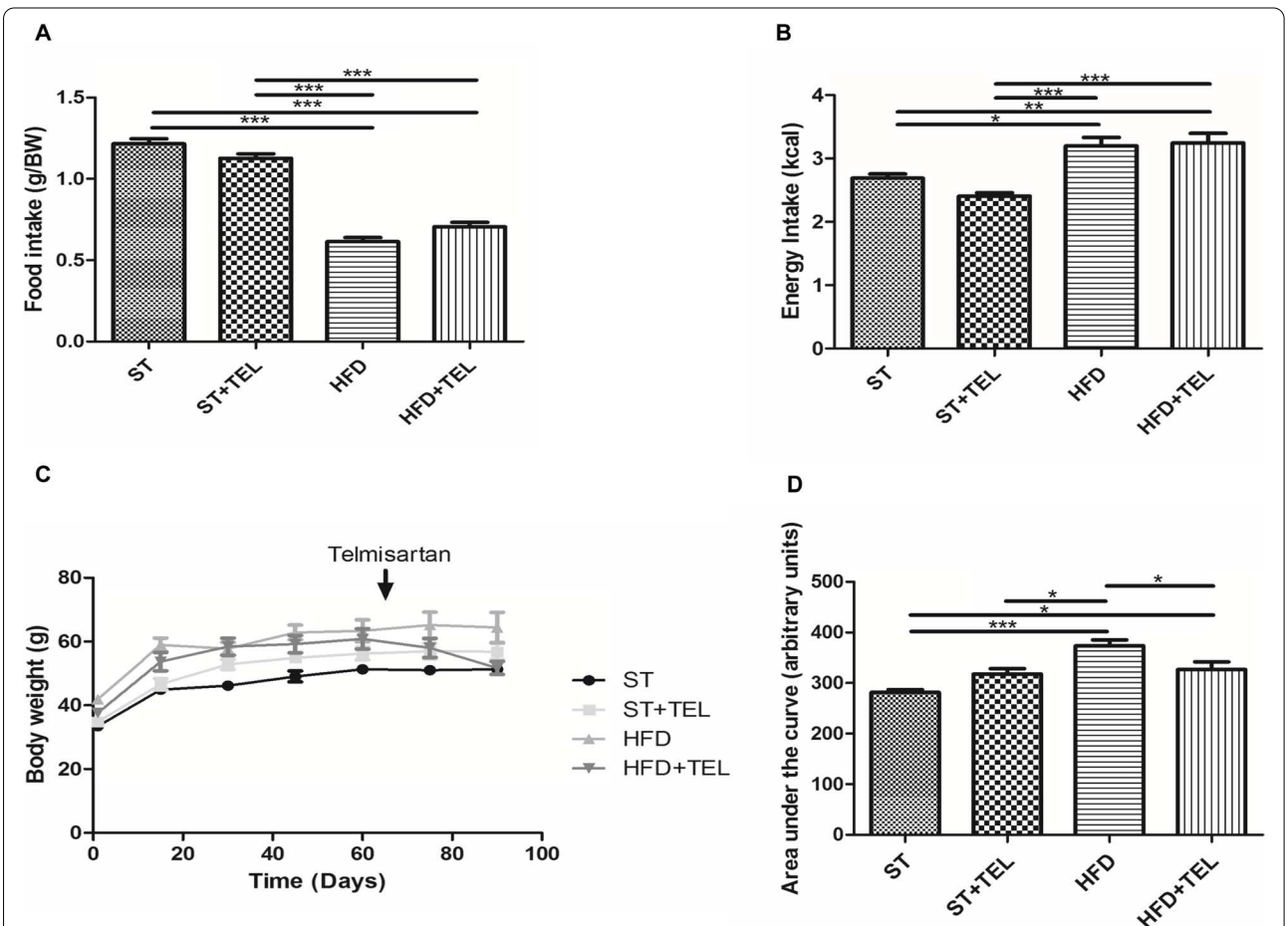


Fig. 1 Food intake, energy intake, and body weight in mice treated with Telmisartan and fed with a standard and high-fat diet (ST, ST+TEL, HFD, HFD+TEL). Food intake (**a**), energy intake (**b**), body weight (**c**), and area under the curve (**d**). Data are presented as mean ± SEM; *p<0.05, **p<0.01, and ***p<0.001

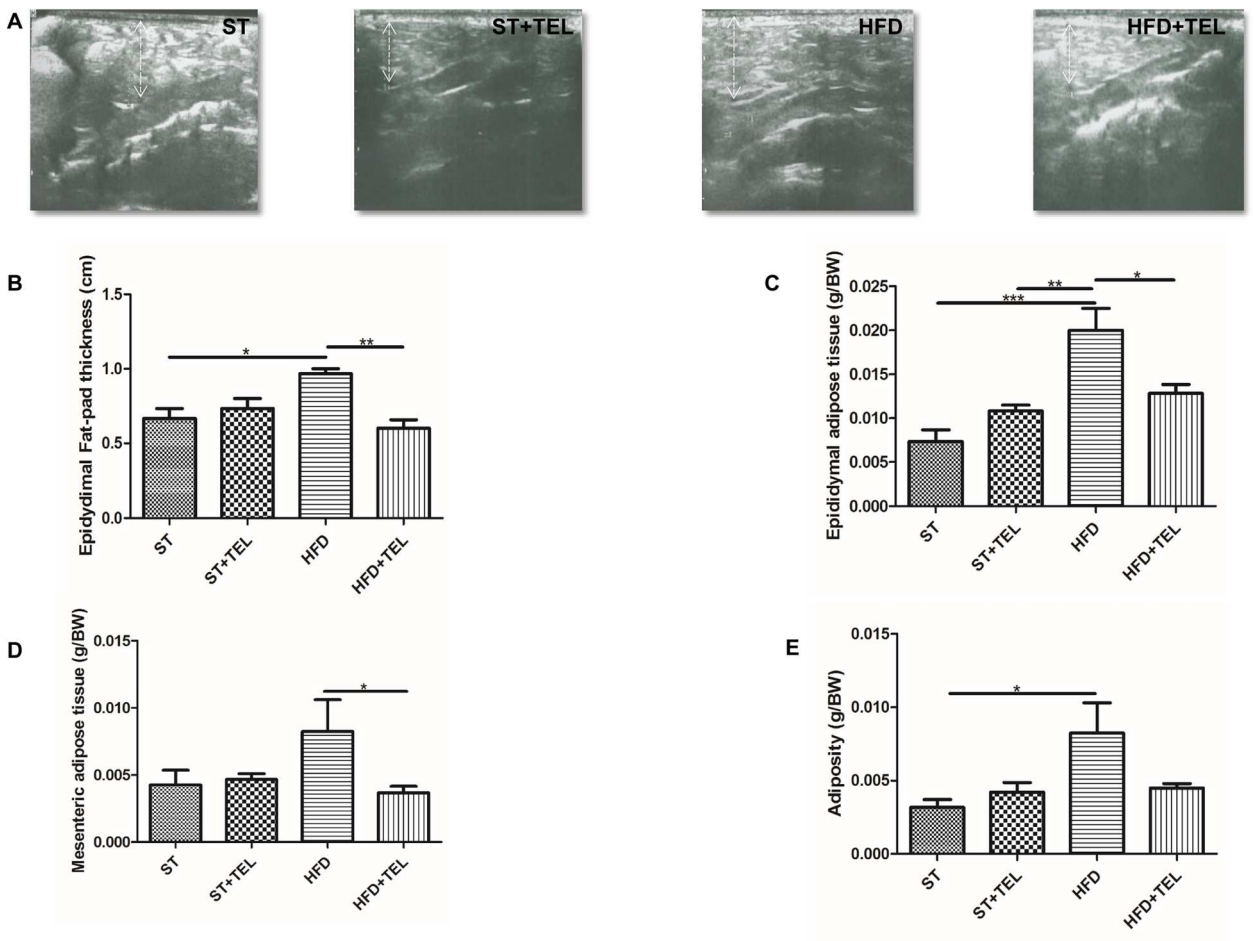


Fig. 2 Adiposity, epididymal, and mesenteric adipose tissues in mice treated with Telmisartan and fed with a standard and high-fat diet (ST, ST+TEL, HFD, HFD+TEL). Epididymal US images (**a**), epididymal fat pad thickness accessed by US (**b**), epididymal adipose tissue weight (**c**), mesenteric adipose tissue weight (**d**), and adiposity (**e**). Arrows in A indicate the epididymal fat pad. Data are presented as mean ± SEM; *$p<0.05$, **$p<0.01$

± 0.15) (Fig. 1a, b). ST animals had lower body weight when compared with HFD animals during all the experiments, and treatment with TEL significantly decreased body weight in the HFD group but not in the ST group (ST:281.4 ± 5.51; ST+TEL:317.5 ± 11.25; HFD:373.6 ± 11.25; HFD+TEL:326.6 ± 15.12) (Fig. 1c, d).

Epididymal fat pad weight was higher in the HFD group when compared with the ST group, and treatment with TEL significantly reduced this weight in the HFD+TEL group (ST:0.007 ± 0.001; ST+TEL:0.01 ± 0.0006; HFD:0.02 ± 0.002; HFD+TEL:0.013 ± 0.001) (Fig. 2c). US measurements of the epididymal fat pad thickness show similar results, with higher measures in HFD animals than in ST animals, and with a significant reduction in epididymal fat pad thickness in the HFD+TEL group in relation to the HFD group (ST, 0.667 ± 0.0667; ST+TEL, 0.733 ± 0.066; HFD, 0.967 ± 0.033; HFD+TEL, 0.600 ± 0.058) (Fig. 2a, b).

Mesenteric adipose tissue also had significantly reduced weight in the HFD + TEL group when comparing with the HFD group (ST, 0.004 ± 0.001; ST+TEL, 0.005 ± 0.0004; HFD, 0.08 ± 0.02; HFD+TEL, 0.004 ± 0.0004) (Fig. 2d). Total adiposity was higher in the HFD group in comparison with the ST groups and was significantly reduced in the HFD+TEL group when compared with the HFD group (ST, 0.013 ± 0.001; ST+TEL, 0.016 ± 0.001, HFD, 0.031 ± 0.002; HFD+TEL, 0.018 ± 0.001 (Fig. 2e).

A low-glucose tolerance was observed in HFD mice when compared with STD animals, and this parameter was significantly improved in the HFD + TEL group (ST, 27314 ± 3393; ST+TEL, 32355 ± 1214; HFD, 49454 ± 2439; HFD+TEL, 39802 ± 1401) (Fig. 3a). Although insulin sensitivity test showed this same tendency, no statistical significance was observed (Fig. 3b). Triacylglycerols did not significantly differ between the groups (ST, 111.8 ± 6.29; ST+TEL, 125.7 ± 10.49;

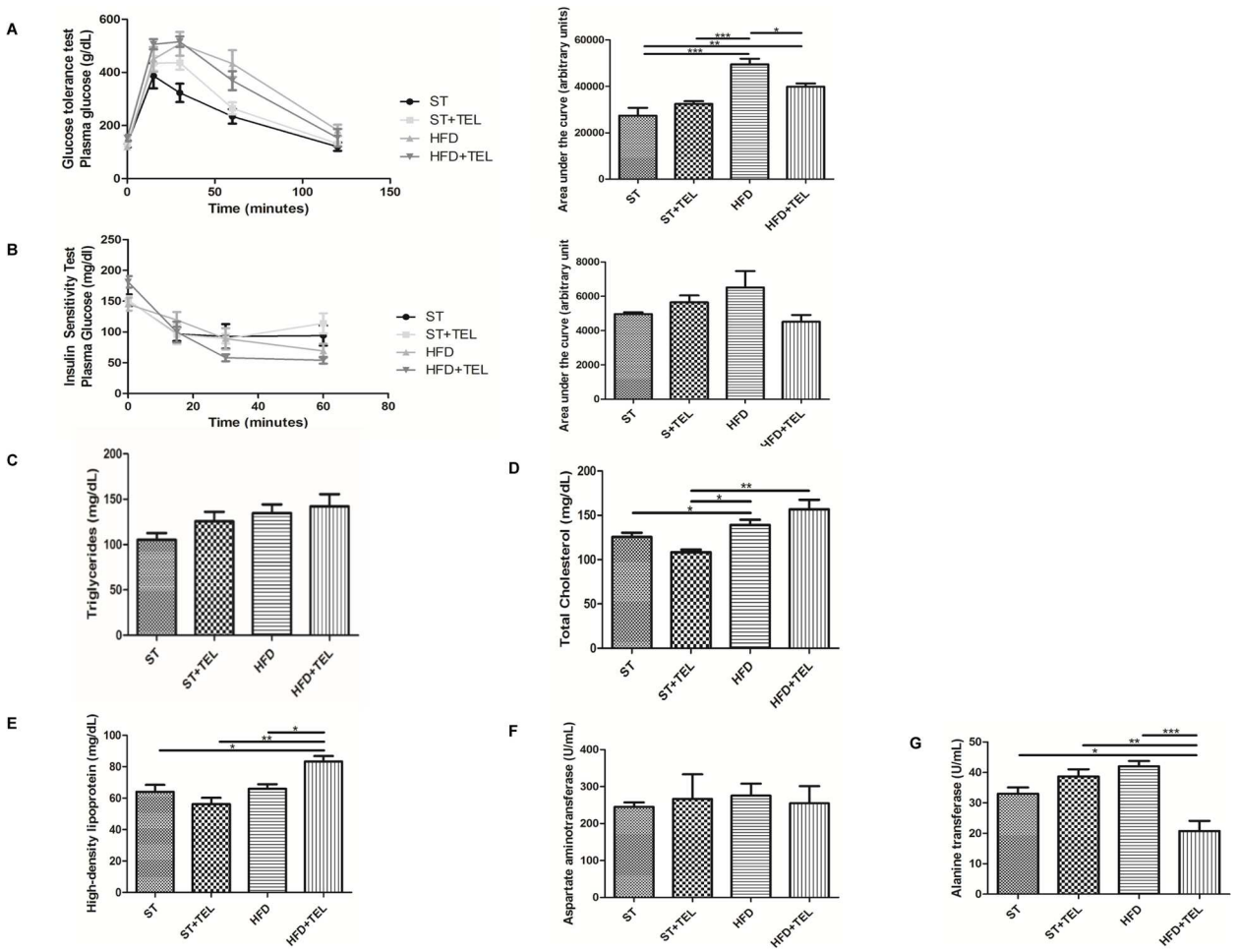


Fig. 3 Insulin sensitivity and glucose tolerance tests, lipid parameters, and hepatic enzymes in mice treated with Telmisartan and fed with a standard and high-fat diet (ST, ST+TEL, HFD, HFD+TEL). Glucose tolerance test (**a**), insulin sensitivity test (**b**), triglycerides (**c**), total cholesterol (**d**), high-density lipoprotein (**e**), aspartate aminotransferase (**f**), and alanine transferase (**g**). Data are presented as the mean ± SEM; **p*<0.05, ***p*<0.01, and ****p*<0.001 versus the group indicated

HFD, 128.3 ± 9.304; HFD+TEL, 142.0 ± 13.53) (Fig. 3c). The HFD+TEL group had higher concentrations of HDL cholesterol than the ST and HFD groups (ST, 64.08 ± 4.40; ST+TEL, 56.20 ± 4.02; HFD, 65.98 ± 2.94; HFD+TEL, 83.33 ± 3.44) (Fig. 3e). Regarding parameters related to liver damage, values of ALT (ST, 33.0 ± 2.1; ST+TEL, 38.67 ± 2.40; HFD, 42 ± 1.79; HFD+TEL, 20.75 ± 3.35; $P = 46.56$) were significantly reduced in the HFD+TEL group (Figs. 3f, g and 4).

In addition, the analysis indicated that the HFD group had a substantial increase in total liver weight in relation to ST mice and that HFD+TEL significantly reduced this weight (ST, 1.833 ± 0.105; ST+TEL, 1.889 ± 0.07; HFD, 2.233 ± 0.012; HFD+TEL, 1.405 ± 0.065) (Fig. 5). US examination of liver size demonstrated higher measurements of the liver longitudinal axis in HFD when comparing with ST animals, with a substantial decrease in the HFD+TEL group in relation to the HFD group (ST, 1.467 ± 0.088; ST+TEL, 2.000 ± 0.200; HFD, 2.233 ± 0.088; HFD+TEL, 1.467 ± 0.120) (Fig. 5a, b). US analysis of liver echogenicity showed elevated mean gray values in the HFD group, with a significant reduction of this parameter after TEL treatment in the HFD+TEL group when compared with HFD animals (ST, 78.68 ± 3.894; ST+TEL, 97.1 ± 5.556; HFD, 130.9 ± 0.012; HFD+TEL, 94.27 ± 9.199) (Fig. 5a–c). This result was subjectively observed by the radiologist during the exam by an increase in the brightness of the hepatic parenchyma in the HFD group and a decrease in this parameter in the HFD+TEL group (Fig. 5a). The histologic analysis displayed steatosis in the hepatocytes of mice fed a high-fat diet as compared to the

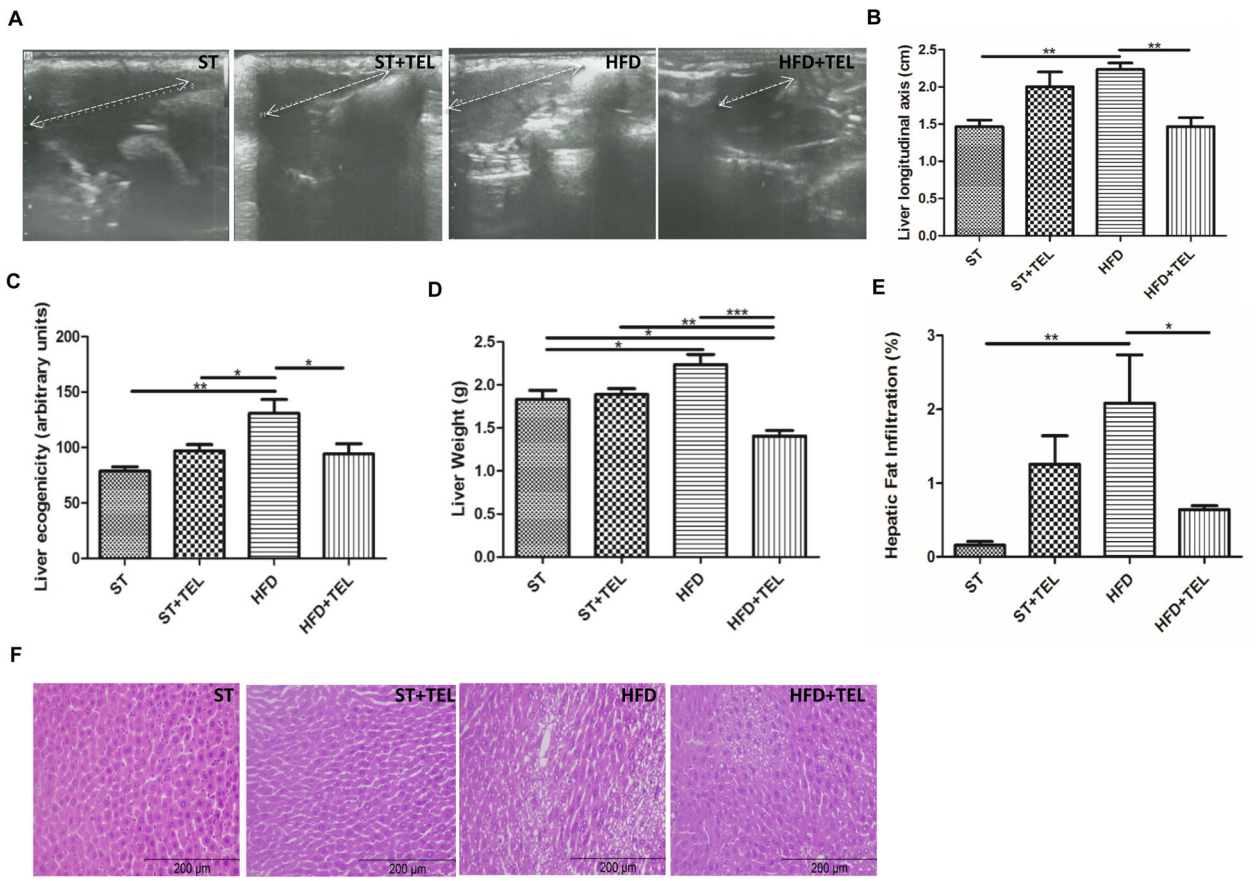


Fig. 4 Liver ultrasonography (US), weight, and hematoxylin/eosin staining in mice treated with Telmisartan and fed with a standard and high-fat diet (ST, ST+TEL, HFD, HFD+TEL). Scale bar, 200 µm. Liver US images (**a**), longitudinal axis measured by US (**b**), echogenicity (**c**), weight (**d**), fat infiltration (**e**), and hematoxylin/eosin staining (**f**). Arrows in A indicate the liver. Data are presented as the mean ± SEM; *$p<0.05$, **$p<0.01$, and ***$p<0.001$ versus the group indicated

standard-fed animals (Fig. 5f). The study also evaluated the effect of telmisartan in the hepatocyte's fat accumulation in obese mice, revealing that TEL significantly reduced the fat accumulation in the high-fat + TEL-treated animals (ST, 0.159 ± 0.049; ST+TEL, 1.253 ± 0.388; HFD, 2.080 ± 0.659; HFD+TEL, 0.642 ± 0.050) (Fig. 5e, f).

The HFD+TEL group significantly reduced the expression of resistin (HFD, 2699± 6071; HFD+TEL, 1203±1128, of TRL4 (HFD, 1.347±0.242; HFD+TEL, 0.153±0.028) and of Myd88 (HFD, 7.630±0.756; HFD+TEL, 0.220±0.137) when compared to the HFD control group (Fig. 5a–c).

Discussion

The present study evaluated the Telmisartan (TEL) efficacy on improving NAFLD in obese mice by modulating resistin, TRL4, and Myd88 inflammatory signaling expression. Despite all the advances in NAFLD/NASH pathophysiology, it is important to emphasize that until today there is no consolidated pharmacological treatment for this hepatic condition, especially considering evidence-based medicine protocols [29].

The main results of the present study showed that TEL effectively protects obese mice against NAFLD and ameliorates several metabolic parameters. The results are in consonance with previous studies which pointed to TEL as the most promising ARB for NAFLD treatment in terms of both safety and efficacy [30]. The pathological changes found in NAFLD may help to understand these findings considering that this hepatic disorder is associated with the renin-angiotensin-system (RAS) inflammatory arm activation [10]. NAFLD is commonly linked to obesity and insulin resistance, which leads to liver fat import and export imbalance [31]. Other relevant cytokines are altered producing an imbalance between pro- and antioxidant actions and pro- and anti-inflammatory effects, which may contribute to the NAFLD

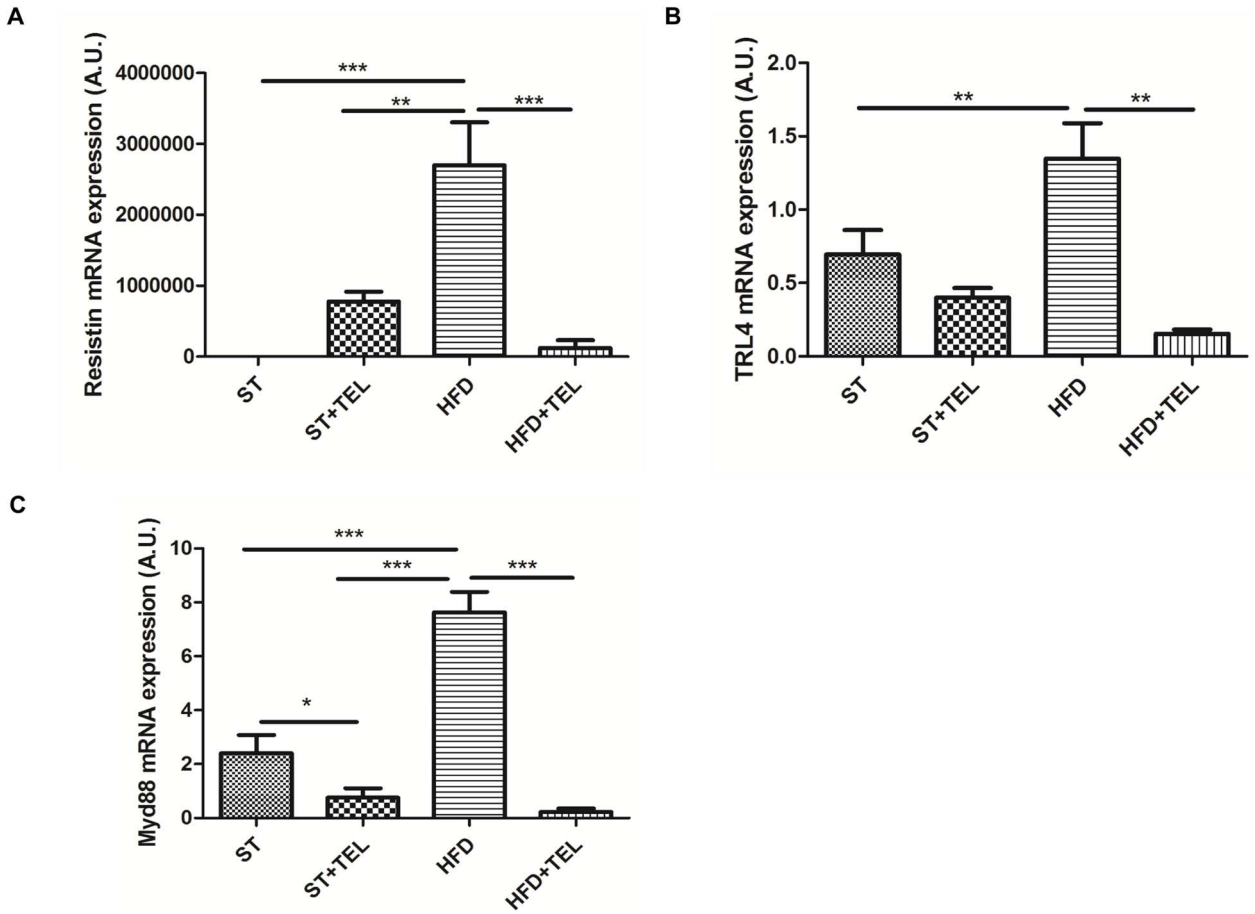


Fig. 5 Liver resistin/toll like-4 (TRL-4) and myeloid differentiation primary response 88 (Myd88) mRNA expression performed by qRT-PCR analyses (**a**, **b**, **c**). Data are presented as the mean ± SEM; *$p<0.05$, **$p<0.01$, and ***$p<0.001$ versus the group indicated

progression to aggravated liver disease such as NASH and fibrosis [32].

The present data demonstrated that HFD mice present a worse glucose profile when compared with ST animals, with a lower glucose tolerance, which was significantly improved by TEL treatment. Concerning insulin sensitivity, a similar tendency was observed despite no statistical significance, which might be explained by the relatively short experiment duration.

The renin-angiotensin system (RAS) has been faced as a two-arms balance with opposite effects on the body composition. Several disease processes are able to unbalance this system. The ACE2/Ang-(1–7)/Mas axis is the antagonist arm with protective effects counteracting the deleterious effects of ACE/Ang II/AT1R axis excessive activation [33]. Evidence suggests that obesity activates the inflammatory RAS arm composed (ACE/Ang II/AT1R) [34] pointing to Angiotensin II (Ang II) as a key piece in the abnormal hepatic lipid metabolism observed in NAFLD [35], which modifies intracellular insulin signaling by several mechanisms that ultimately result in worsening of insulin resistance. Ang II also induces the generation of reactive oxygen species (ROS), that initiates and propagates the pro-inflammatory mediator's production, such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and plasminogen activator inhibitor-1 (PAI-1), resulting in inflammation and additional impairment of insulin signaling, creating a vicious cycle of steatosis, necroinflammation, and fibrosis [31]. On the other hand, increasing evidence has shown the beneficial effects of the ACE2/Ang-(1-7)/Mas axis on liver function and metabolism, with an important anti-obesity role in improving insulin sensitivity, glucose tolerance, and type 2 diabetes; modulating body fat; increasing adiponectin production; and reverting hyperleptinemia [32, 36].

Concerning AT1R-independent effects, it is important to emphasize the relationship between TEL and peroxisome proliferator-activated receptor gamma (PPAR-**γ**) activation. The nuclear PPARγ receptor is a transcription factor that regulates genes related to adipogenesis,

lipid metabolism, and insulin sensitivity. TEL, unlike other ARBs, can work as a partial agonist of PPAR-**γ** [13], increasing insulin sensitivity, high-density lipoprotein levels, and decreasing inflammation, oxidative stress, cell proliferation, migration, fatty acid, and triglyceride levels. This effect occurs without the side effect of liquid retention produced by full PPAR-γ agonists such as pioglitazone or rosiglitazone [37].

TEL not only modulates RAS in order to inhibit AngII/AT1R deleterious effects, but also increases the activity of ACE2/Ang-(1-7)/Mas arm [33], beyond its PPAR-**γ** partial agonistic effects [34] and has been consistently proven to reduce insulin resistance [12]. We demonstrated a significant improvement in liver size and hepatic lipid droplet infiltration in the HFD group treated with TEL, in accordance with previous studies [13]. The morphological findings were positively correlated with the liver ultrasound (US) exam on the day before the sacrifice. Ultrasound was effective in demonstrating a higher liver size in the HFD group and a substantial decrease in this parameter when the HFD animals were treated with TEL. Also, US analysis of liver echogenicity showed elevated mean gray values in the HFD group, with a significant reduction of this parameter after TEL treatment in the HFD+TEL group. As in humans, hepatic steatosis in rodents is classically described on US as a diffuse increase in hepatic echogenicity, or "bright liver," due to increased reflection of US from the liver parenchyma, which is caused by intracellular accumulation of fat vacuoles [35]. Another parameter, such as reduced visualization of the diaphragm and of small peripheral vessels, with no changes in the liver surface, can also be utilized [38].

We also demonstrated that TEL reduces the adiposity and the mesenteric and epididymal adipose tissue weights in the HFD+TEL group when compared with the HDF group. Liao et al. [37] used measurements of epididymal fat pad thickness echography to successfully access the amount of this adipose tissue deposition. Kudo et al. [39] showed for the first time that TEL decreases the adipocyte size and upregulates the adiponectin secretion without affecting food intake in a murine NASH model, reducing the accumulation of visceral fat. Moreover, TEL but not the ARB Valsartan, increased the expression of both nuclear-encoded and mitochondrial-encoded genes in the skeletal muscle known to play important roles in mitochondrial energy metabolism.

Thus, in addition to a class effect of ARBs in modulating adipocyte size, the present findings raise the possibility that TEL may have a particularly strong impact on fat cell volume and fat accumulation and stand out in its metabolic effects, which may help to protect against dietary-induced visceral obesity and weight gain. The efficacy of TEL in reducing visceral fat mass may be relevant for patients since an increase in visceral fat is related to hypertension, dyslipidemia, and an impaired metabolic pattern, but also works as an independent predictor of mortality in humans [40].

Telmisartan effectively reduced liver expression of the pro-inflammatory resistin/TRL4/MYD88 pathway in HFD animals. Resistin is an important adipokine in an obesity setting and is positively correlated with increased fat mass and with a proinflammatory state, as reported in chronic liver diseases [41]. Resistin modulates the synthesis and secretion of essential proinflammatory cytokines such as TNF-α and IL-6 by an NF-κB-dependent pathway [42]. Santos et al. [43] showed that rats with HFD-induced obesity presented increased hepatic expression of resistin/TRL4/MAPK/NF-KB pathway developing insulin resistance, glucose intolerance, hyperinsulinemia, and dyslipidemia.

Toll-like receptors (TRLs) are expressed in immune cells and works in the immunological response to microbial agents. The activation of TRLs induces antimicrobial pathways of the innate defense, upregulation of antigen-presentation molecules, and secretion of cytokines that influence adaptive immune response. Inappropriate activation of TRL pathways by endogenous or exogenous ligands may lead to the initiation and/or perpetuation of autoimmune responses and tissue damage [14]. It has recently been reported that the TRL4 signaling pathway plays an important role in the progression of hepatic inflammation and fibrosis. This effect occurs by triggering the expression of proinflammatory cytokines through mydd88-dependent activation that in turn is mediated by interleukin receptor-associated kinase IL-1 (IRAK), factor 6 associated with tumor necrosis factor receptor (TRAF6) and kinase 1 activated by transforming growth factor 1, which activates IkB kinase (IKK), leading to transcription of the NF-kB [44]. Alquami et al. [45] corroborated these findings by demonstrating that rats fed with a high-fructose diet showed hepatic TRL4 overexpression.

The antifibrotic and hepatoprotective effects of TEL in different animal models [38] and human have been reported in several studies. TEL has been shown to have hepatoprotective effects on NAFLD decreasing hepatic fibrosis via its antioxidant and anti-inflammatory activity by preventing NF-kB signaling pathway stimulation [45]. Similar results were described in humans were TEL reduced hepatic oxidative stress and fibrogenesis in patients with NAFLD and chronic hepatitis C [1].

Telmisartan reduced liver damage resulting from type I diabetes mellitus [38] and when associated with propranolol reduced the liver fibrosis signs in a murine model of primary sclerosing cholangitis (PSC) [46]. Yi et al. showed that TEL was also able to prevent liver fibrosis

in a rat bile duct ligation model [47]. In mice fed with a high-fat diet treated with streptozotocin (STZ) low dose (2 days after birth), treatment with TEL reduced liver inflammation and fibrosis [48]. Similarly, TEL reduced short- or long-term diet-induced hepatic fibrosis in rats [49] preventing hepatocellular carcinoma [50]. The anti-inflammatory activity of TEL can be partly explained by the RAS blockade (AngII/AT1R), which plays an important role in the induction and maintenance of inflammation and oxidative stress [51–53].

Conclusion

In conclusion, the present study indicates that oral treatment with Telmisartan offers a protective effect against liver damage in NAFLD and improves lipid and glucose metabolism in high-fat fed mice. Telmisartan effectively reduced hepatic pro-inflammatory resistin/TRL4/MYD88 expression in obese animals, which could explain, at least in part, the positive effects on improving liver fat deposition.

Abbreviations

ARB: Angiotensin type I receptor blocker; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GAPDH: Endogenous glyceraldehyde 3-phosphate dehydrogenase; HDL: High-density protein; HE: Hematoxylin and eosin; HFD: High-fat diet; IKK: IkB kinase; IL-1: Interleukin-1; IL-6: Interleukin-6; IRAK: Interleukin receptor-associated kinase IL-1; LDL: Low-density protein; MD2: Myeloid differentiation factor 2; MetS: Metabolic syndrome; MyD88: Myeloid differentiation factor 88; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; NF-κb: Nuclear factor-κB; PAI-1: Plasminogen activator inhibitor-1; PPAR-γ: Peroxisome proliferator-activated receptor gamma; PSC: Primary sclerosing cholangitis; SD: Standard deviation; ST: Standard diet; TEL: Telmisartan; TNF-α: Tumor necrosis factor-alpha; TRAF6: Tumor necrosis factor receptor; TRLs: Toll-like receptors; RAS: Renin-angiotensin-system; ROS: Reactive oxygen species; STZ: Streptozotocin.

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Authors' contributions

Conception, design, acquisition of data, analysis and interpretation of data, and write the manuscript: LMAB, DFF, ASM, AFP, and BVC: Luciana Mendes Araújo Borém, Daniela Fernanda de Freitas, Amanda Souto Machado, Alanna Fernandes Paraíso, and Bruna Viana Caldas; writing and critical review of the manuscript: João Felício Rodrigues Neto, Juliana Pinto Lima, André Luiz Sena Guimarães, and Alfredo Maurício Batista de Paula; conception, design, analysis, and interpretation of the data and writing of the manuscript: Sérgio Henrique Sousa Santos. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information.

Declarations

Ethics approval and consent to participate

The animal studies were previously approved under number: 022/2012 by the ethical committee CEEBEA - Unimontes.

Consent for publication

The authors approved the publication process conducted by the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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