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Protective effects of pitanga leaf extract on oxidative stress and hepatic-testicular alterations in a rat model of metabolic syndrome

Efek protektif ekstrak daun pitanga terhadap stres oksidatif dan perubahan liver dan testis pada model tikus sindrom metabolik

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Abstract

The prevalence of metabolic syndrome (MetS) is increasing. Oxidative stress is a major consequence of MetS and can affect multiple organs, including the liver and testes. Pitanga leaves possess potent antioxidant and anti-inflammatory properties and are rich in anthocyanins, phenolic acids, and flavonoids, which may provide protection against oxidative damage. This study evaluated the effects of pitanga leaf extract on oxidative stress markers and liver and testicular histology in a rat model of MetS. Twenty-five male Wistar rats were randomly assigned to five groups: normal, MetS, MetS + telmisartan (8 mg/kg), MetS + pitanga leaf extract (PLE) 50 mg/kg), and MetS + PLE (100 mg/kg). MetS was induced for eight weeks using a high-fat diet, and 20% fructose was administered concurrently with the interventions. Plasma oxidative stress markers were assessed on day 57, after which the tissues were collected for histological examination. Administration of PLE at 50 and 100 mg/kg reduced plasma malondialdehyde levels and increased glutathione peroxidase levels ($p < 0.05$). It also improved liver and testicular indices ($p < 0.05$) and reduced hepatic steatosis. In conclusion, pitanga leaf extract ameliorated oxidative stress and liver and testicular damage in rats with MetS, although its testicular effect was limited to reducing the testicular index.

Keywords: *Eugenia uniflora*, testicular histology, liver histology, oxidative stress, metabolic syndrome

Abstrak

Prevalensi sindrom metabolik semakin meningkat. Stres oksidatif merupakan konsekuensi utama MS dan memengaruhi berbagai organ, termasuk liver dan testis. Daun dewandaru memiliki aktivitas antioksidan dan antiinflamasi yang kuat. Dibandingkan antioksidan lain, daun dewandaru kaya senyawa antosianin, asam fenolik, dan flavonoid yang memberikan perlindungan lebih luas terhadap kerusakan oksidatif. Penelitian ini bertujuan mengevaluasi aktivitas ekstrak daun dewandaru terhadap penanda stres oksidatif, histologi liver, dan testis pada tikus model MS. Studi eksperimental dilakukan pada 25 tikus Wistar jantan yang dialokasikan secara acak menjadi 5 kelompok: normal, MS, MS+telmisartan 8 mg/kg, MS+ekstrak daun dewandaru (PLE) 50 mg/kg, dan MS+PLE 100 mg/kg. Selama delapan minggu, diet tinggi lemak dan fruktosa 20% diberikan untuk menginduksi MS, bersama dengan perlakuan. Penanda oksidatif plasma dinilai pada hari ke-57, dan jaringan diambil untuk pemeriksaan histologis. Pemberian PLE 50 dan 100 mg/kg memperbaiki stres oksidatif pada tikus model MS yang ditunjukkan dengan penurunan MDA plasma dan peningkatan kadar glutation peroksidase ($p < 0.05$). Ekstrak ini juga memperbaiki indeks liver dan testis ($p < 0.05$) serta mengurangi steatosis hati. Kesimpulan, ekstrak daun dewandaru memperbaiki stres oksidatif dan kerusakan hati serta testis pada tikus model MS, namun efeknya pada testis terbatas pada penurunan indeks testis.

Kata Kunci: *Eugenia uniflora*, histologi testis, histologi hepar, stres oksidatif, sindrom metabolik

Introduction

Metabolic syndrome (MS) is an assemblage of risk factors, including obesity, increased blood sugar, elevated triacylglycerol levels, decreased HDL cholesterol, and hypertension (Dobrowolski et al., 2022; Fahed et al., 2022). This condition not only doubles the risk of cardiovascular disease, stroke, and type 2 diabetes but also increases the risk of non-alcoholic fatty liver disease, polycystic ovary syndrome, organ failure, reproductive issues, and obstructive sleep apnea (Mohamed et al., 2023). Approximately 20–30% of individuals worldwide experience MS (Belete et al., 2021). The prevalence of MS is predicted to increase alongside rising obesity rates, sedentary lifestyles, and excessive consumption of calorie-dense foods and sugar-sweetened beverages (Fahed et al., 2022).

Increased systemic pro-inflammatory cytokines and advanced glycation end-products are prominent consequences of MS and can affect multiple organs, including the liver and testes. Elevated lipid peroxidation, reactive oxygen species (ROS) production, and oxidative DNA damage have been closely linked to MS in male animal models. Additionally, MS components are negatively correlated with sperm motility and morphology, leading to impaired testicular function and sperm DNA fragmentation in patients with MS (Marchiani et al., 2015). Animals with a high-fat diet (HFD)-induced MS exhibit reduced testicular weight, lower antioxidant activity (superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px)), and increased susceptibility to pro-oxidative damage (Billah et al., 2022). On the other hand, MS may also cause hepatic oxidative damage, decreased fatty acid oxidation, and elevated triglyceride synthesis, often coexisting with non-alcoholic fatty liver disease (NAFLD) (Fahed et al., 2022).

Pitanga, (*Eugenia uniflora L., Myrtaceae*), or dewandaru, is a tropical plant known for its antioxidant potential (de Brito et al., 2022; Fidelis et al., 2022). Most previous studies have focused on the fruits, whereas the leaves, which contain higher concentrations of phenolic acids and flavonoids, remain underexplored (Fidelis et al., 2022). This represents a significant research gap, as pitanga leaves may provide greater antioxidant efficacy and organ protection. Given the rising prevalence of MS and associated oxidative organ damage, exploring locally sourced functional ingredients is critical. Pitanga's abundant leaf shows promising pharmacologic qualities, while the fruit is in limited supply; therefore, this study aimed to evaluate the effects of pitanga leaf extract (PLE) on oxidative stress markers and histological alterations in the liver and testes of rats with MS.

Methods

This true experimental laboratory study employed a post-test-only control group design at the Integrated Research Laboratory of the Islamic University of Indonesia from January to May 2025. Twenty-five male *Rattus norvegicus* rats from a previous study on PLE and MS were used in this study. Ethical clearance was obtained from the Ethics Committee of the Faculty of Medicine, Islamic University of Indonesia (No. 8/Ka.Kom.Et/70/KE/X/2024).

The number of subjects was determined using Festing's formula (Arifin & Zahiruddin, 2017). Animals were randomly allocated to five groups: Normal Control, untreated MS (MS Control), MS + Telmisartan 8 mg/kg (MS+T), MS + PLE 50 mg/kg (MS+PLE50), and MS + PLE 100 mg/kg (MS+PLE100).

Preparation of Pitanga Leaf Extract

Approximately 500 g of *E. uniflora* leaves were collected from Sleman District, Yogyakarta, and authenticated at the Laboratory of Plant Systematics, Faculty of Biology, Gadjah Mada University (herbarium register no. 00608/S.Tb/IV/2024). The powdered leaves were macerated in 5 L of 96% ethanol for 3 × 24 h. The filtrate was evaporated to yield 48.05 g of crude extract.

Intervention

MS was induced using a combination of 1 mL oxidized oil, 2 mL quail egg yolk per 100 g BW, and 20% D-fructose in drinking water (Merck Milipore, Indonesia) for 56 d (Aji et al., 2023; Reshidan et al., 2019). Telmisartan (8 mg/kg BW) and PLE (50 or 100 mg/kg BW) were administered via an oral gavage.

On day 57, the animals were sedated using ketamine (25 mg/kg BW, i.p.), followed by retro-orbital blood collection and organ harvesting. The organ indices were calculated as Organ Weight (mg)/BW (g) (Cui et al., 2021).

Biochemical Parameter Measurements

Collected blood samples were centrifuged at 1,500 × g for 15 min to obtain plasma, which was stored at -20°C for later analysis. Plasma malondialdehyde (MDA) was examined using the CheKine™ MDA Assay Kit (Cat. No. KTB1230) using a colorimetric method. The samples were mixed with the reaction reagent and incubated at 95°C for 30 min.

After centrifugation, the supernatants were transferred to a 96-well microplate. Absorbance was measured at 532 nm and 600 nm. The GSH-Px activity was assessed using the FineTest® Rat GSH-Px1 ELISA Kit (Cat. No. ER0274), using a double-antibody sandwich method. After adding samples and detection antibodies, incubation and washing steps were followed by the addition of HRP-streptavidin and TMB substrate. The absorbance was measured at 450 nm.

Histological Preparation and Examination

Liver and testicular tissues were processed into 5 µm paraffin sections and stained with hematoxylin and eosin (HE). Five photomicrographs taken from a digital camera-equipped microscope (Olympus CX22, Japan, and Optilab Viewer, Indonesia) were used to identify the liver and the testicles. Two observers blinded to the intervention performed the observations. Hepatic steatosis (fatty vacuoles) and inflammation were assessed in the liver (Prasomthong et al., 2022).

Liver histopathology was subjected to the intraclass correlation coefficient (ICC) to assess observation validity. The ICC value was 0.96, indicating excellent agreement (ICC > 0.90) (Liljequist et al., 2019). The Consentino classification and interstitial scoring were analyzed in the testicles (Table 1) (Yalçin et al., 2020).

Statistical Analysis

Data were analyzed using GraphPad Prism 9.3.1 and SPSS version 26. Normality was tested using the Shapiro-Wilk test. Parametric data were analyzed using ANOVA with post hoc tests, while non-parametric data were analyzed using Kruskal-Wallis and Mann-Whitney tests. Significance was set at $p < 0.05$.

Table 1: Histopathological score classification

Consentino Classification	Score	Interstitial Tissue	Score
Normal testicular structure with germinal cells arranged in an ordered fashion	1	No Lesion	-
Closely packed seminiferous tubules and less ordered germ cells	2	Slight Lesion	+
Disorganized germinal cells with less pronounced seminiferous tubule orders and smaller pyknotic nuclei	3	Medium-Level Lesion	++
Closely packed seminiferous tubules with coagulative necrosis of the germinal cells	4	Severe Lesion	+++

Result and Discussion

Figure 1 illustrates the effects of various treatments on organ indices and plasma oxidative stress markers across the five groups. The testicular index in the MS control group was significantly lower than that in the normal control group. Administration of telmisartan and PLE at 100 mg/kg significantly increased the testicular index compared to the MS Control group.

The liver index in the MS group was higher than that in the Normal Control group. Treatment with 100 mg/kg PLE resulted in a lower liver index than that in the MS control group. Regarding plasma oxidative stress markers, the MS Control group exhibited significantly elevated plasma MDA levels compared to the Normal Control group.

Rats receiving PLE (50 and 100 mg/kg) showed significantly reduced plasma MDA levels compared to the MS Control group. Although plasma GSH-Px levels in the MS Control group were slightly lower than those in the Normal Control group, the difference was not statistically significant. Treatment with both PLE doses led to a significant increase in GSH-Px activity compared to that in the MS control group.

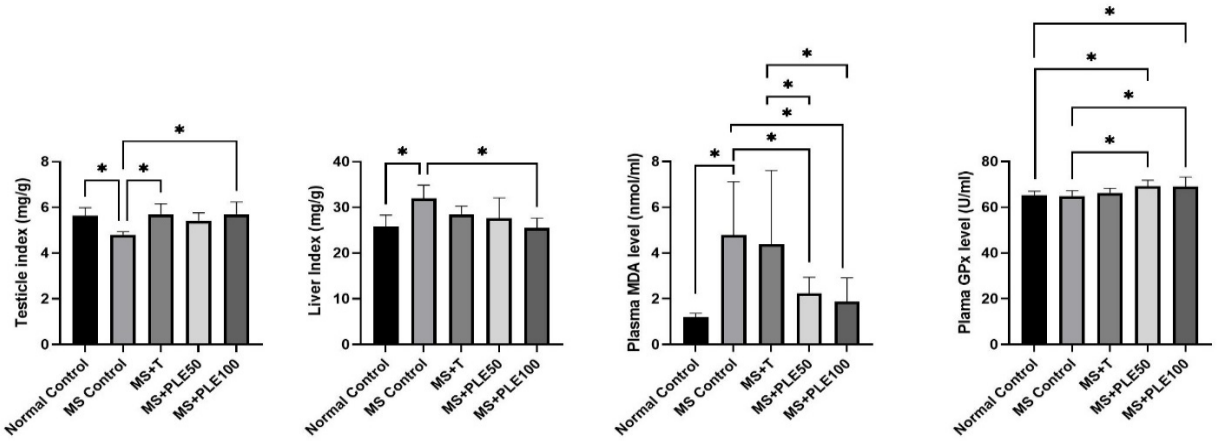
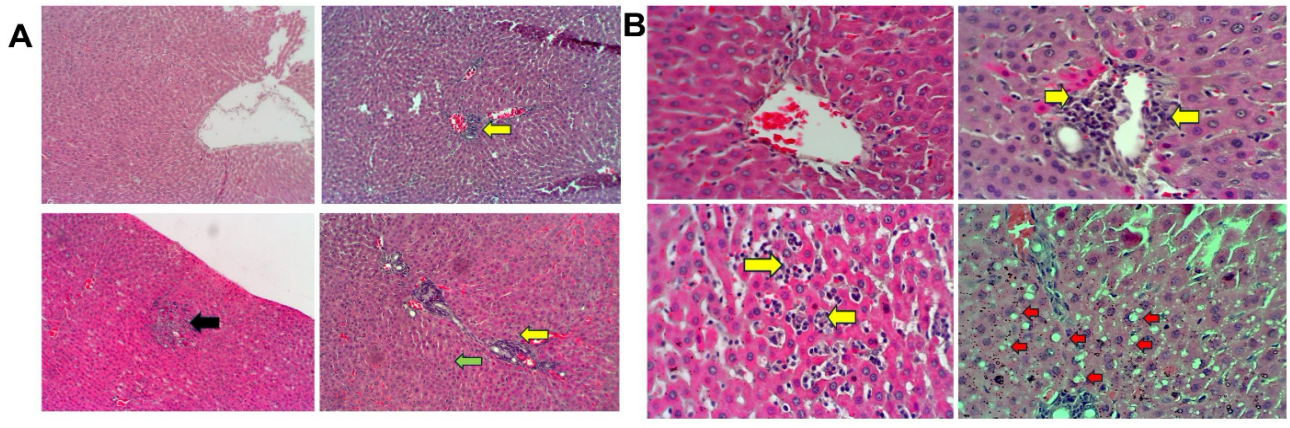


Figure 1. Organ indices and plasma oxidative markers. *p<0.05, compared to the MS group (Anova followed by post-hoc Tukey), n=5

Figure 2 shows the liver histopathological analyses. The normal control group exhibited normal liver architecture without pathological alterations. In contrast, the MS group displayed steatosis and portal inflammation, which was characterized by dense inflammatory cell infiltration.



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Group	Ballooning Degeneration	Intralobular Inflammation	Portal Inflammation	Fibrosis
Normal Control	0	0	++	0
MS Control	+	+	++++	++
MS + T	0	0	+++	+
MS + PLE50	+	+	++	++
MS + PLE100	0	+	++	+

Figure 2. Representative images of liver sections from each group are shown. Data are presented as medians. Kruskal-Wallis and Mann-Whitney tests, n = 5, HE staining.

At lower magnification (100×, Panel A), the normal control liver showed intact hepatic architecture, whereas the MS group exhibited abnormalities, including portal inflammation (yellow arrow), lobular inflammation (black arrow), and fibrosis (green arrow). Detailed views at higher magnification (400×, Panel B) demonstrated normal hepatic tissue with central veins (top left), lymphocyte infiltration in the portal and

lobular areas (yellow arrow), and hepatic steatosis (red arrows).

Panel C shows that pitanga leaf extract (PLE) reduced hepatic steatosis in a dose-dependent manner in a rat model of metabolic syndrome. The highest dose (100 mg/kg) produced the strongest protective effect, potentially exceeding that of telmisartan. Panel D summarizes the qualitative and histopathological findings. At higher magnifications, hepatic ballooning degeneration and intralobular inflammation were observed in the MS group. Telmisartan treatment mitigated these pathological changes, as indicated by reduced steatosis and inflammatory cell infiltration. Both PLE doses exhibited dose-dependent hepatoprotective effects.

While PLE50 moderately reduced steatosis and inflammation, PLE100 substantially improved these parameters. Semi-quantitative scoring of histopathological features reinforced these observations. The MS group exhibited the most severe pathological changes, including prominent ballooning degeneration, intralobular and portal inflammation, and fibrosis. Conversely, telmisartan- and PLE100-treated groups showed marked reductions in these parameters and steatosis scores, indicating that PLE100 is comparable to telmisartan.

Figure 3 shows the histological findings of the testes. Panel A shows the seminiferous tubules of the control group, which were uniformly organized, separated by a narrow interstitial space, and lined with a thick epithelium. In contrast, the MS group (upper middle and right panels) exhibited atrophic epithelium and partial desquamation of seminiferous tubules.

In Panel A, the control group demonstrated optimal epithelial thickness (red arrow, upper left). In the MS group, the middle and upper right seminiferous tubules displayed atrophied and sloughed epithelium (blue arrow), edema, and interstitial hemorrhage (yellow arrow). The MS+T (bottom left) and MS+PLE 50 (bottom center) groups showed disorganized epithelium with minimal interstitial changes (yellow arrow). The MS+PLE100 group (bottom right) demonstrated a marked structural improvement.

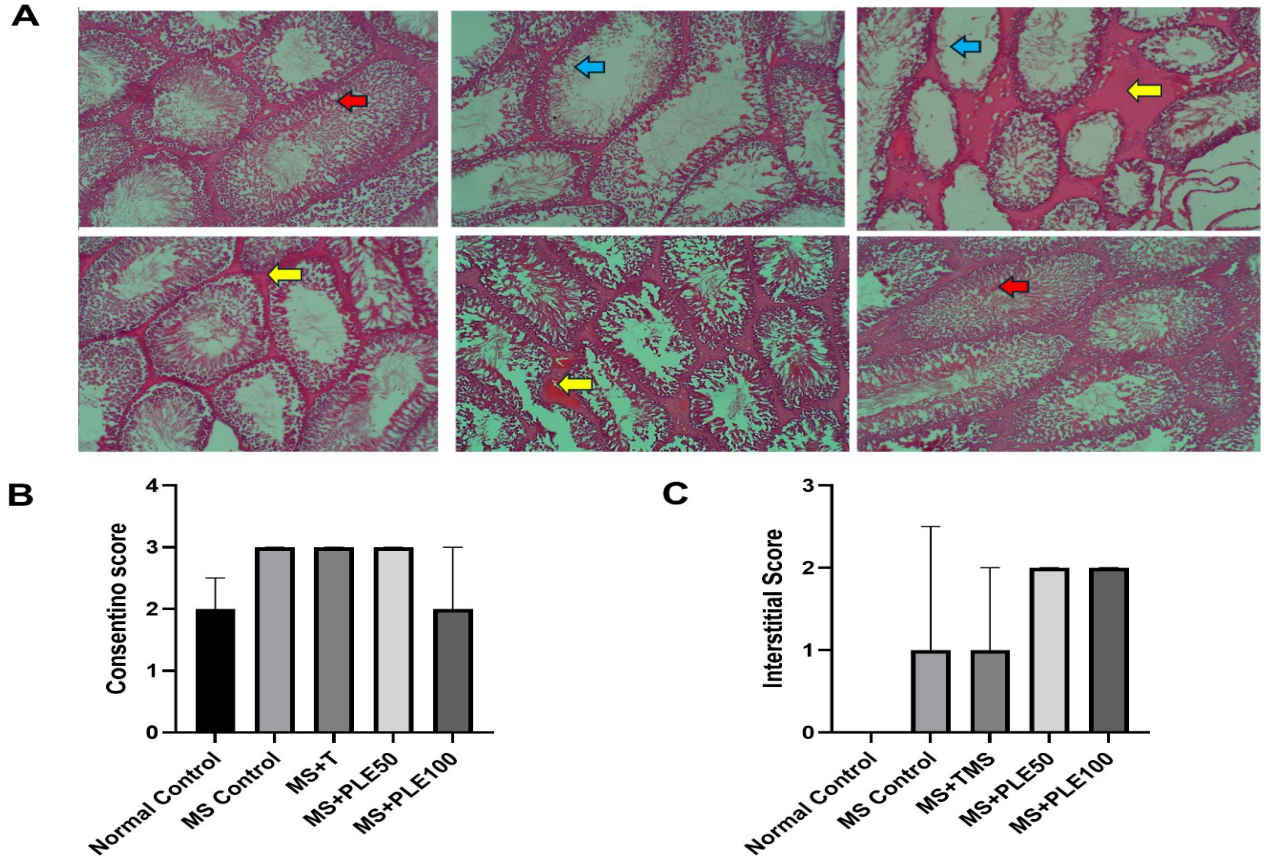

Figure 3. Photomicrograph of the testis. Data are presented as medians. Kruskal-Wallis and Mann-Whitney tests n = 5, HE staining. 100X.

Panels B and C show the Consentino and interstitial scores. The MS group exhibited reduced epithelial thickness, cell detachment, increased intertubular distance, interstitial edema, and minor hemorrhage. Telmisartan and PLE50 partially improved these changes, but residual edema and hemorrhage remained, whereas PLE100 showed thicker seminiferous epithelium with minimal interstitial abnormalities. However, non-parametric analyses revealed no significant differences in Consentino and interstitial scores among groups.

A combination of HFD and fructose in drinking water for 8 weeks successfully induced oxidative stress, as indicated by a significant increase in plasma MDA levels in the MS group. This finding aligns with previous studies that reported that a 12-week HFD increases cholesterol and MDA levels in rats (Hazzaa et al., 2021). Similarly, an 8-week HFD elevates plasma MDA levels and induces hepatic steatosis (Lasker et al., 2019). High fructose intake for 12 weeks has also been shown to increase plasma MDA levels, likely due to elevated body weight, triglyceride, and cholesterol levels, thereby promoting lipid peroxidation (Chávez-Gutiérrez et al., 2022).

A high-fat diet (HFD) not only increases plasma MDA levels but also induces inflammation, as indicated by elevated hs-CRP levels (Anionye & Otasowie, 2024). Although the GSH-Px activity was slightly lower in the MS group than in the normal controls, the difference was not statistically significant, consistent with previous reports showing that combined HFD and high-sugar diets increase oxidative stress without significantly altering GSH-Px activity (Kobi et al., 2023). However, another study reported HFD induces oxidative stress in rat adipose tissue, accompanied by decreased GSH-Px activity, suggesting that alternative mechanisms may be involved (Charradi et al., 2013).

The development of ROS, DNA damage, and elevated lipid peroxidation are indicative of oxidative damage to sperm and testes and are closely associated with male models of MS. Additionally, MS is negatively correlated with testicular function and is associated with decreased androgen levels, which can lead to reductions in spermatozoa count, epididymal integrity, Sertoli cell density, germ cell degeneration, and depletion of the seminiferous epithelium (Marchiani et al., 2015).

The liver index was higher in untreated MS animals, and histopathological analysis revealed liver damage, including steatosis, inflammation, and fibrosis. Similarly, HFD-induced MS models exhibit hepatocyte vacuolization, sinusoidal dilatation, and increased inflammatory mediators (Albrahim & Alonazi, 2021). Elevated free fatty acid levels in MS can exacerbate liver metabolic dysregulation and insulin resistance, leading to chronic inflammation and oxidative stress (Fahed et al., 2022). Triglyceride accumulation in hepatocytes, ballooning degeneration, and lipopolysaccharide-stimulated proinflammatory cytokine production are key features of steatosis (Leow et al., 2023). Moreover, lipopolysaccharides will stimulate the production of pro-inflammatory cytokines and will trigger fibrogenesis (Radu et al., 2023).

Pitanga leaf extract improved oxidative stress parameters and liver damage, but its testicular protection was limited to maintaining the testicular index. Both PLE doses were equally effective in reducing MDA levels and increasing GSH-Px activity, comparable to telmisartan. These findings align with previous reports of the antioxidant and hepatoprotective properties of pitanga fruit and leaf extracts (Santoso et al., 2021; Fajeria, 2020; de Brito et al., 2022; Sobeh et al., 2020).

Flavonol glycosides from the leaves exhibit antioxidant, and anti-inflammatory activities that may underlie increased GSH-Px levels (DeLaney et al., 2019). Phytochemical analyses confirm multiple bioactive compounds with the ethyl acetate fraction showing the highest antioxidant activity (Olugbuyiro et al., 2018). Consistently, flavonoid-rich ethanol extracts demonstrate superior antioxidant effects in TBARS and ROS assays compared with aqueous extracts (Bagatini et al., 2023). Mechanistically, the antioxidant effects of *E. uniflora* may involve the modulation of the Nrf2/Keap1 pathway, enhancing the transcription of antioxidant defense genes, and suppressing NF-κB-mediated inflammation (Masenga et al., 2023).

Notably, pitanga extract (100 mg/kgBW) showed a protective effect comparable to or exceeding telmisartan, a standard regimen for MS. In contrast, telmisartan-treated MS rats exhibited persistently elevated MDA levels, with no significant difference from untreated MS controls, suggesting limited direct antioxidant effects (Fujita et al., 2012). Mechanistically,

telmisartan acts via AT1R blockade and partial PPAR-γ activation, improving insulin sensitivity and reducing NADPH oxidase–mediated ROS production (Wahba et al., 2025). However, its antioxidant effects appear context-dependent, as other studies report reductions in MDA and increased SOD (Rodríguez-Lara et al., 2019). In the present study, GSH-Px levels were modestly increased but not significantly, aligning with evidence of enhanced GSH-related antioxidant activity in certain tissues (Almukainzi et al., 2024). Overall, telmisartan remains beneficial in MS, partly through modulation of PPAR pathways (Cheng et al., 2018).

This study has several limitations. Phytochemical analysis of pitanga leaf extract was not performed, precluding identification of the active constituents. Second, telmisartan was commercially sourced, which may affect purity and comparability. Additionally, organ protection was evaluated only structurally; inclusion of functional or molecular assessments would provide a more comprehensive evaluation.

Conclusion

Pitanga leaf extract reduced plasma oxidative stress markers and ameliorated hepatic injury, with 100 mg/kgBW being the most effective dose. The protective effects on the testicular structure were limited. Further studies are needed to elucidate the molecular mechanisms and identify the active compounds.

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